Clavulanic Acid and its Derivatives. Structure Elucidation of Clavulanic Acid and the Preparation of Dihydroclavulanic Acid, Isoclavulanic Acid, Esters and Related Oxidation Products

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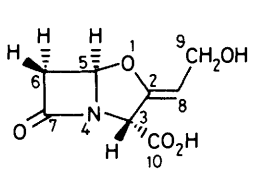
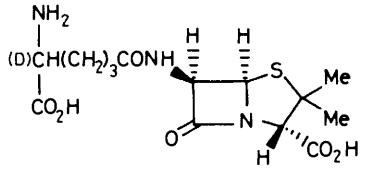
Clavulanic acid, a novel β-lactamase inhibitor from *Streptomyces clavuligerus*, has been shown to be *Z*-(2*R*,5*R*)-3-(β-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (**1**). The structure and absolute stereochemistry was confirmed by *X*-ray analysis of the *p*-nitrobenzyl and *p*-bromobenzyl esters, (**14**) and (**15**). The conversion of clavulanic acid into a variety of esters and acyl derivatives is described. An account of its isomerisation to the *E* isomer (**30**) and the formation of an oxetane (**45**) as a by-product of the photolysis of the phenacyl ester (**18**) is given. The reduction and oxidation of acid (**1**) under a variety of conditions has also been examined in detail.

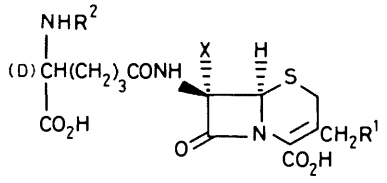
Streptomyces clavuligerus (ATCC 27064, NRRL 3585) has been shown to produce the novel β-lactamase inhibitor clavulanic acid (MM 14151) (**1**)[1-3] and to provide the β-lactam antibiotics penicillin N (**2**),[4] the cephalosporins (**3**)[5] and (**4**),[6] and the cephamycins (**5**)[6] and (**6**).[7] Recently, further clavam derivatives (**7**)—(**11**) have been reported from *S. clavuligerus* mutants[8,9] and another Streptomyces species,[10] while clavulanic acid (**1**) has also been isolated from *S. jumojinensis*,[11] *S. katsurahamanus*,[12] and *Streptomyces sp.* P6621.[13] In addition to these β-lactam antibiotics *S. clavuligerus* produces holomycin and MM 19290.[14] In this paper we describe the structure elucidation of clavulanic acid in detail and outline some of its chemistry. Preliminary accounts of some of these studies have been presented earlier.[3,15]



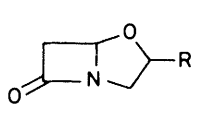
(**1**)



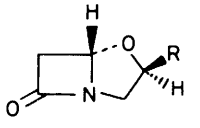
(**2**)



(**3**) $R^1 = R^2 = X = H$
(**4**) $R^1 = OCONH_2$; $R^2 = X = H$
(**5**) $R^1 = OCONH_2$; $R^2 = H$; $X = OMe$
(**6**) $R^1 = OCONH_2$; $R^2 = CH_2CONH_2$; $X = OMe$



(**7**) $R = CH_2OH$
(**8**) $R = CH_2OCHO$



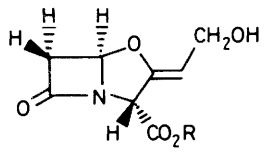
(**9**) $R = CO_2H$
(**10**) $R = CH_2CHCO_2H$ (NH_2)
(**11**) $R = CH_2CH_2OH$

By the application of a screening procedure[1,2] designed to detect β-lactamase inhibitors in culture filtrates, it was found that *S. clavuligerus* produced a metabolite, clavulanic acid (originally designated as MM 14151). Independent of these studies other workers have also obtained the β-lactam (**1**) from the same source.[16]

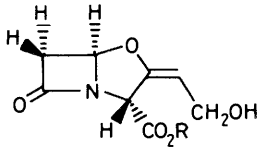
Alkylation of sodium clavulanate (**12**) with methyl iodide in dimethylformamide gave a methyl ester (**13**) whose molecular formula $C_9H_{11}NO_5$ was established by mass spectral data and elemental analysis. In the mass spectrum a base peak at *m/z* 196, derived by loss of OH $[M - OH]^+$ was typical of an allylic alcohol function, while a fragmentation $[M - C_2H_2O]^+$ corresponded to a loss of ketene, a cleavage pattern characteristic of β-lactams.[17] The i.r. spectrum of the ester (**13**) contained an intense absorption band at 1 800 cm^{-1}, assigned to a β-lactam carbonyl, in addition to an ester carbonyl band at 1 750 cm^{-1} and absorption at 1 695 cm^{-1} ascribed to the strained exocyclic double bond.

The β-lactam protons in the n.m.r. spectrum were evident as an ABX system; the 6β-H was a double doublet [δ 3.05 (*J* 17.5 and 0.8 Hz)] as was the 6α-H [δ 3.54 (*J* 17.5 and 2.8 Hz)] and the 5α-H [δ 5.72 (*J* 2.8 and 0.8 Hz)]. The small *trans* (0.8 Hz) and *cis* (2.8 Hz) coupling constants for these protons relative to those for penicillanic acid derivatives is explained by the effect of replacing sulphur with oxygen in the clavam ring system and may also indicate a distortion of the azetidinone fragment of this fused β-lactam ring system.[18] The β-hydroxyethylidene unit was present as an AA'X system in which the C-9 methylene protons absorbed at δ 4.24 (dd, *J* 7.2 and 6.5 Hz) and the adjacent C-8 methine proton absorbed at δ 4.93 (ddd, *J* 7.2, 6.5, and 1.2 Hz) and showed further allylic coupling to the C-3 proton. As a result of coupling with the C-8 proton (*J* 1.2 Hz) and the long-range coupling with the C-9 protons the C-3 proton appeared as a broad doublet (δ 5.07). These assignments were supported by decoupling studies. The ^{13}C n.m.r. showed the appropriate resonances for nine carbon atoms with the double bond carbons C-2 and C-8 at 152 and 100 p.p.m. respectively. The chemical shift of the C-5 carbon (87 p.p.m.) was downfield from the analogous C-5 resonance of penicillanic acid derivatives, while the C-6 carbon (46 p.p.m.) had a similar shift to that of the C-6 carbon in methyl penicillanate.

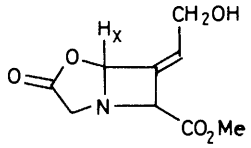
The above evidence was consistent with the proposed structure (**13**) for methyl clavulanate or the double bond isomer (**27**). A possible alternative non-β-lactam structure (**32**) was considered, which is in agreement with some of the spectrosopic



(12) R = Na
(13) R = Me
(14) R = $CH_2C_6H_4NO_2$-*p*
(15) R = $CH_2C_6H_4Br$-*p*
(16) R = $CH_2C_6H_4OMe$-*p*
(17) R = CH_2Ph
(18) R = CH_2COPh
(19) R = $(CH_2)_8Me$
(20) R = CH_2OCOBu^t
(21) R = Phthalidyl
(22) R = Phthalimidomethyl
(23) R = Ph_2CH
(24) R = Ph
(25) R = 2-(2-Pyridyl)ethyl
(26) R = CH_2CCl_3



(27) R = Me
(28) R = $CH_2C_6H_4Br$-*p*
(29) R = CH_2Ph
(30) R = Na
(31) R = CH_2COPh



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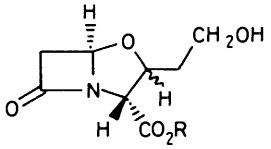
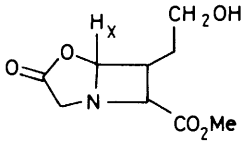


(33) R = Me
(34) R = CH_2Ph



(35)

properties above, however, this was readily eliminated on the basis of the n.m.r. data of methyl dihydroclavulanate (**33**).

Reduction of the double bond of methyl clavulanate (**13**) in ethyl acetate with 10% Pd/C as catalyst was complete after 2 days and yielded an epimeric mixture (2:1) of methyl dihydroclavulanates which were separated by h.p.l.c. The major epimer was confirmed as ester (**33**) from n.m.r. spectroscopy data which showed that the X part (δ 5.72) of the ABX system of the β-lactam protons had moved upfield (to δ 5.25) by 0.47 p.p.m. while the multiplicity (dd) of this proton remained as in the starting ester (**13**). Reduction of the alternative structure (**32**) would lead to a product (**35**) where the multiplicity of the proton (H_X) would now be expected to be different from that of the starting material (**32**).

The structure and relative stereochemistry of clavulanic acid were confirmed by *X*-ray analysis of the *p*-nitrobenzyl ester (**14**) and this established the configuration of the double bond as *Z*. The absolute configuration was determined *via* the *p*-bromobenzyl ester (**15**) which showed that the chirality of the C-5 and C-3 protons was identical with that of the naturally occurring penicillins, and unambiguously established clavulanic acid (**1**) as *Z*-(2*R*,5*R*)-3-(β-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.*

Fractional atomic co-ordinates, bond-lengths and -angles for compounds (**14**) and (**15**) are given in Tables 1—6 (see p. 647 *et seq.*) and the conformation in the crystal lattice is illustrated in the perspective drawing (Figure 1). The structure of (**15**) as revealed by the *X*-ray work (see Experimental section) was not of the highest accuracy. The number of observations was too small to justify either refinement of the light atoms anisotropically or the inclusion of hydrogen atoms. The *R*-value of 8.8% with these restrictions is quite respectable. A detailed discussion of the geometry of the molecule is unwarranted but all lengths and angles were within two standard deviations of accepted values. For bond lengths the extremes of the standard deviations were 0.02 and 0.06 Å with the great majority between 0.03 and 0.04 Å. For angles the range was 1—3°.

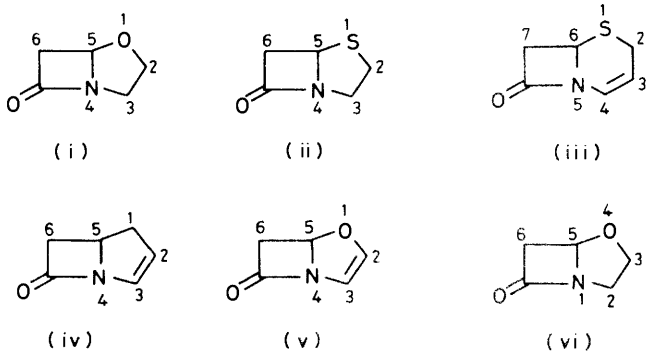
The determination of the absolute configuration was made by two methods. The initial stages of the refinement were carried out without including anomalous dispersion factors in the calculations. At convergence anomalous dispersion was introduced and the co-ordinates of *both* enantiomers were loaded into the computer on cards. This precaution was taken so that the hypothesis to be tested by Hamilton's statistical method was strictly one dimensional. The ratio of the weighted *R*-factors under these conditions was 1.018 (0.0929/0.0912), significant at much below the 0.005 level.

Additionally a comparison was made of the relative intensities of Friedel pairs (measured consecutively). Calculations showed that in 45 cases the intensity difference between the members of the pairs should be greater than the scatter of the count. In 44 of these cases the expected significant difference was observed. In the remaining case the members of the pair showed almost the same intensity.

Both procedures gave the same assignment of configuration and we thus allocate the 3*R*,5*R* configuration to the ester with complete confidence.

In addition to the methyl, *p*-nitrobenzyl, and *p*-bromobenzyl esters of clavulanic acid esters, such as the benzyl (**17**), phenacyl (**18**), *p*-methoxybenzyl (**16**), nonyl (**19**), pivaloyloxymethyl (**26**), and phthalimidomethyl (**22**) were prepared by alkylation using the appropriate halide in dimethylformamide (DMF) at room temperature. In this manner reaction of sodium clavulanate with 3-bromophthalide gave a diastereoisomeric mixture of phthalidyl esters (**21**) which were separated by h.p.l.c. Treatment of sodium clavulanate with 2,2,2-trichloroethyl chloroformate in dry tetrahydrofuran gave after chromatographic purification a low yield of 2,2,2-trichloroethyl clavulanate (**26**). The methyl (**13**), phenyl (**24**), pyridylethyl (**25**), and *p*-tolylthio (**36**) esters were prepared *via* clavulanic acid in

* Clavulanic acid is a derivative of 4-oxa-1-azabicyclo[3.2.0]heptan-7-one which has also been given the trivial name clavam (i) by analogy with the nomenclature used for other fused β-lactam ring systems such as penam (ii), ceph-3-em (iii), and 1-carbapen-2-em (iv). In this paper the majority of compounds are described using clavam (i) and clavem (v) nomenclature with the numbering of the atoms following the sequence used for penams (*e.g.* in penicillins) and ceph-3-em (*e.g.* in cephalosporins). It should be noted that when compounds are described as derivatives of the 1-azabicyclo[3.2.0]heptane ring numbering starts from the bridgehead nitrogen, as indicated in (vi).



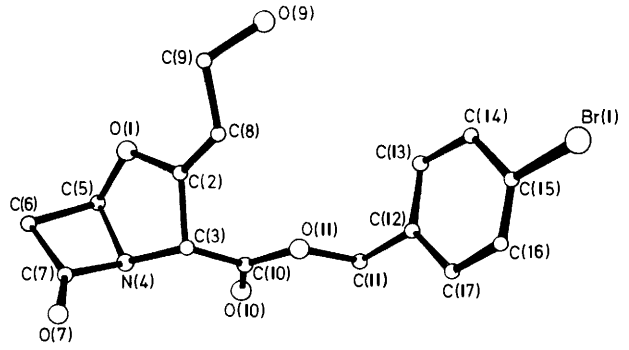
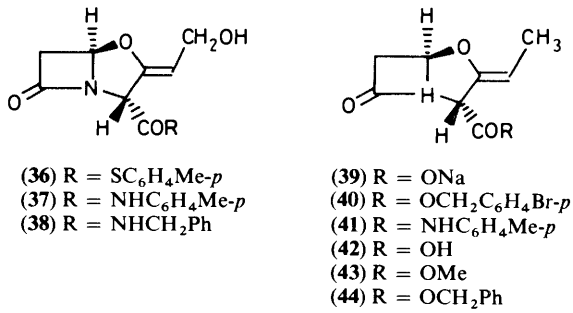


Figure 1. Perspective view of *p*-bromobenzylclavulanate (**15**)



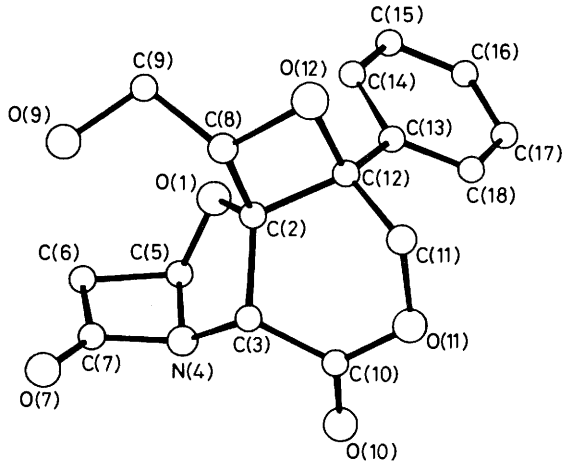


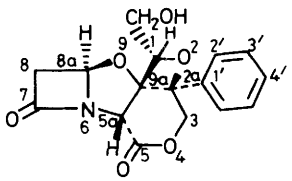
Figure 2. Perspective view of the oxetane (**45**)

acetonitrile containing dicyclohexylcarbodi-imide and the corresponding alcohol or thiol; the methyl (**13**) and benzhydryl (**23**) esters could also be obtained by addition of diazomethane or diphenyldiazomethane to an ethanolic solution of clavulanic acid.

In order to determine the full chemotherapeutic potential of clavulanic acid derivatives it was necessary to utilise a C-3 ester function that could be readily converted into the corresponding free acid or carboxylate. The benzyl, *p*-nitrobenzyl, and methyl ester functions were found to be most suitable for such conversions, hydrogenolysis of the esters (**17**) or (**14**) being utilised while the methyl ester (**13**) was readily hydrolysed under conditions of controlled pH to yield appropriate alkali-metal salts. This ready hydrolysis of an alkyl ester of clavulanic acid contrasts with the renowned poor susceptibility to hydrolysis exhibited by simple alkyl esters of penicillins and cephalosporins.[19]

As already indicated dihydroclavulanic acid derivatives can be prepared by catalytic hydrogenation. Further clavulanic acid derivatives can be obtained with hydrogen and heterogeneous catalysis depending on a variety of factors such as solvent, reaction time, pH, ratio of substrate to catalyst, and rate of agitation. Hydrogenation of benzyl clavulanate (**17**) over palladised charcoal in aqueous ethanol containing sodium hydrogen carbonate until the theoretical 1 mole equivalent of hydrogen had been absorbed gave pure sodium clavulanate (**12**) readily isolated as the crystalline tetrahydrate. If the hydrogenation, however, was allowed to proceed under uncontrolled conditions involving an excess of hydrogen uptake due to longer reaction times, certain by-products were obtained. These products could be isolated and characterised *via* ester formation. Thus, more prolonged exposure of the ester (**17**) to catalytic hydrogenation in aqueous ethanol led to a mixture of two compounds which were separated *via p*-bromobenzyl ester formation and chromatography. One of the esters obtained was the expected compound (**15**) while the other proved to be *p*-bromobenzyl isoclavulanate (**28**) as shown by n.m.r. and mass spectroscopy data and confirmed by *X*-ray crystallographic analysis (Tables 10—12; see page 649.). Hydrogenolysis of the ester (**17**) had therefore led to the preparation of the *E*-isomer of clavulanic acid. A similar reaction on benzyl clavulanate (**17**) but with increased exposure (3 days) to hydrogen led to an even more complex mixture of products. After esterification to produce the methyl esters of these products, methyl clavulanate (**13**), methyl isoclavulanate (**27**), and methyl dihydroclavulanate (**33**) were isolated, while a further compound was identified as methyl deoxyclavulanate (**43**); a small quantity of methyl dihydrodeoxyclavulanate was also considered (by t.l.c.) to have been formed during this reaction. Benzyl clavulanate (**17**), on hydrogenolysis in tetrahydrofuran with an equal weight of palladised charcoal as catalyst and followed by sodium hydrogen carbonate treatment, was converted into sodium deoxyclavulanate (**39**), this structure being established by preparation of the ester (**40**). When this reaction was repeated in the absence of alkali the intermediate acid (**42**) was transformed into the amide (**41**) using dicyclohexylcarbodi-imide and *p*-toluidine. Again hydrogenation of the ester (**17**) in ethyl acetate solution over platinum oxide gave benzyl dihydroclavulanate (**34**) as an equimolar mixture of C-2 epimers.

The double-bond isomerisation of clavulanic derivatives (*Z*-isomers) into isoclavulanic analogues (*E*-isomers) was also achieved by u.v. irradiation (low pressure).[20] For example, irradiation of a benzene solution of ester (**17**) gave a photoequilibrium mixture (2:1) of benzyl isoclavulanate (**29**) and starting material (**17**); again catalytic hydrogenolysis of (**29**) led to sodium isoclavulanate (**30**). When irradiation of phenacyl clavulanate (**18**) was carried out in benzene solution, the expected phenacyl isoclavulanate (**31**) was formed, along with a crystalline isomeric compound (**45**), which appeared to contain an ester or lactone function ($\nu_{max.}$ 1 700 cm^{-1}) and a β-lactam carbonyl group ($\nu_{max.}$ 1 800 cm^{-1}) but lacked an absorption band at 1 690 cm^{-1} ascribed to the tri-substituted double bond of the starting material (**18**). *X*-Ray analysis of this product established its structure to be that of the tetracyclic β-lactam lactone (**45**). The fractional atomic co-ordinates, bond-lengths and bond-angles for β-lactam (**45**) are given in Tables 7—9 (see page 648 *et seq.*) and a perspective drawing (Figure 2) depicts its conformation in the crystal lattice. It would appear that this oxetane (**45**) is derived by the coupling of radical intermediates formed from the exocyclic double bond and the phenacyl carbonyl group of the ester (**18**).[21]



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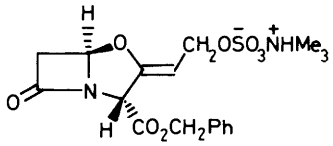

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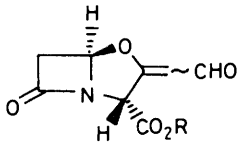
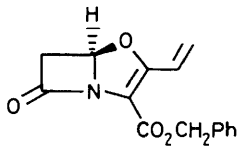


(47) R = CH_2Ph
(48) R = Me
(49) R = $CH_2C_6H_4OMe$-*p*



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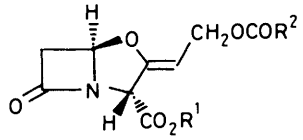
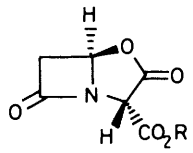
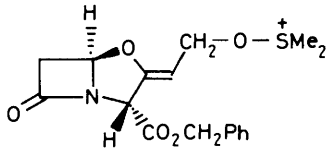


(51) R^1 = CH_2Ph, R^2 = CH_2OPh
(52) R^1 = R^2 = CH_2Ph
(53) R^1 = CH_2Ph, R^2 = $CHPh(CO_2CH_2Ph)$
(54) R^1 = CH_2Ph, R^2 = $CHPh(CO_2Ph)$
(55) R^1 = Phthalimidomethyl, R^2 = $CHPh(CO_2CH_2Ph)$
(56) R^1 = CH_2Ph, R^2 = Me
(57) R^1 = CH_2Ph, R^2 = CH_2Cl
(58) R^1 = CH_2Ph, R^2 = $CHCl_2$
(59) R^1 = CH_2Ph, R^2 = NH_2
(60) R^1 = CH_2Ph, R^2 = NHMe
(61) R^1 = CH_2Ph, R^2 = NHPh
(62) R^1 = CH_2Ph, R^2 = NHCOPh
(63) R^1 = CH_2Ph, R^2 = $NHCOCCl_3$
(64) R^1 = H, R^2 = CH_2OPh
(65) R^1 = Me, R^2 = CH_2Ph
(66) R^1 = Na, R^2 = CH_2OPh
(67) R^1 = H, R^2 = NHMe
(68) R^1 = Na, R^2 = NHMe
(69) R^1 = H, R^2 = CH_2Ph
(70) R^1 = Me, R^2 = CH_2Ph
(71) R^1 = H, R^2 = $CHPh(CO_2H)$
(72) R^1 = Me, R^2 = $CHPh(CO_2Me)$
(73) R^1 = Na, R^2 = Me



(74) R = $CH_2C_6H_4Br$-*p*
(75) R = CH_2Ph



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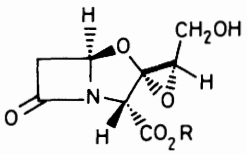
C-3 Amide derivatives (**37**) and (**38**) of clavulanic acid were readily prepared using the appropriate amine and carbodi-imide.

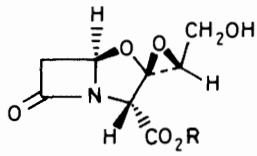
The allylic alcohol function of clavulanic acid was acylated using a variety of methods. Treatment of benzyl clavulanate (**17**) with (i) an acid in the presence of dicyclohexylcarbodi-imide and pyridine, (ii) an acid chloride in pyridine, (iii) with a mixed anhydride and base, or (iv) with an isocyanate resulted in the formation of the range of acyl derivatives (**51**)—(**63**). Deprotection of such acyl compounds to generate the corresponding free acid and/or sodium salt was readily achieved by hydrogenolysis. For example acids (**67**), (**69**), and (**71**), and the related salt (**68**), and derivatives (**70**), and (**72**) were obtained by reaction with hydrogen in tetrahydrofuran solution employing 10% palladised charcoal as catalyst. As with simple clavulanate esters hydrogenolysis of acyl derivatives led to a range of products depending on the solvent used. Benzyl 9-*O*-phenoxyacetylclavulanate (**51**) or benzyl 9-*O*-(*N*-methylcarbamoyl)-clavulanate (**60**) on catalytic hydrogenolysis in ethyl acetate or ethanol formed sodium deoxyclavulanate (**39**) on basic work-up, while under similar conditions in tetrahydrofuran solution benzyl 9-*O*-phenoxyacetylclavulanate (**51**) could be converted into the corresponding free acid (**64**) and sodium salt (**66**) and subsequently the ester (**65**). Direct acetylation of sodium clavulanate (**12**) with acetic anhydride at room temperature produced sodium 9-*O*-acetylclavulanate (**73**). A sulphate ester (**46**) of clavulanic acid could be prepared by treatment of the ester (**17**) with trimethylamine–sulphur trioxide complex.

Oxidation of the allylic alcohol function of clavulanic acid with a number of reagents led to a variety of clavam derivatives.[22] When the ester (**17**) was oxidised using pyridinium chlorochromate the aldehyde (**47**) was obtained as an inseparable (1:1.2) mixture of *E*- and *Z*-isomers as judged by n.m.r. data. The isomers were distinguished by the ^1H n.m.r. chemical shifts of the aldehydic and olefinic protons; the deshielding effect of the ring oxygen atom results in a downfield shift (*ca.* 0.5 p.p.m.) of the olefinic proton resonance in the *E*-isomer, compared to that in the *Z*-isomer. Similarly, the ^1H n.m.r. shift of the aldehydic proton in the *Z*-isomer appears *ca.* 0.3 p.p.m. downfield from that in the *E*-configuration. With the methyl (**13**) and the *p*-methoxybenzyl (**16**) esters a similar reaction occurred yielding (**48**) and (**49**); the aldehydes (**47**) and (**49**) could also be formed *via* manganese dioxide oxidation of (**17**) and (**16**). The aldehydes were extremely unstable to silica gel chromatography resulting in isolated yields of only 5—15%. In an attempt to convert the ester (**17**) into the aldehyde (**47**) under Pfitzner-Moffatt conditions (dimethyl sulphoxide–dicyclohexylcarbodi-imide–orthophosphoric acid in benzene)[23] the product obtained was not the anticipated (**47**) but the diene derivative (**50**). It would appear that the optically active diene (**50**) is formed *via* a 1,4-elimination process from an intermediate sulphonium salt (**76**) in preference to a 1,2-elimination to aldehyde (**47**). Similarly esters of clav-2-em (**50**) have been obtained by base catalysed 1,4-elimination from *O*-acyl clavulanates, *e.g.* (**58**) and from 9-chloro derivatives.[24] Ozonolysis of clavulanate esters (**15**) and (**17**) gave the bicyclic lactones (**74**) and (**75**); the reaction to give (**74**) was of importance in biosynthetic studies using labelled precursors in order to determine labelling patterns in clavulanic acid.[25]

Benzyl clavulanate (**17**) reacted with *m*-chloroperbenzoic acid to give an oxidation product which, although unstable to silica gel, could be substantially purified by chromatography on cellulose. The product possessed a molecular ion at *m/z* 305 in the mass spectrum, indicating the addition of one oxygen atom and implying epoxidation of the double bond. The ^1H n.m.r. spectrum was consistent with the formation of two isomeric epoxides (**78**) and (**80**) (*ca.* 1.2:1), the isomer ratio being



(77) R = $CH_2C_6H_4OMe$-*p*
(78) R = CH_2Ph



(79) R = $CH_2C_6H_4OMe$-*p*
(80) R = CH_2Ph

determined by the intensities of the 2-CH resonances (δ 4.46 and 4.69). *p*-Methoxybenzyl clavulanate (**16**) was similarly converted into the epoxides (**77**) and (**79**).

Experimental

M.p.s were determined on a Kofler hot-stage apparatus and are uncorrected. I.r. spectra were recorded on a Perkin-Elmer 157 spectrometer. ^1H N.m.r. spectra were measured at 60 MHz on Perkin-Elmer R12A or at 90 MHz on Perkin-Elmer R32 instruments and ^{13}C measurements were obtained using a Varian CFT 20 spectrometer; except where otherwise stated spectra were obtained in $CDCl_3$ for esters with tetramethylsilane as internal standard or in D_2O for salts using the HOD peak at δ 4.6 as internal standard. Chemical shifts (^1H and ^{13}C) are quoted in δ values (p.p.m.) relative to the reference and where trivial nomenclature is used are assigned according to the numbering system shown in formula (**1**). Mass spectra were obtained on an AEI MS 9 spectrometer operating at 70 eV. Analytical and preparative t.l.c. were carried out on Merck precoated silica gel 60 F_{254} glass plates which were visualised by u.v. light and/or aqueous potassium permanganate spray and/or Ehrlich's reagent; [26] t.l.c. was carried out routinely on all reaction mixtures and final products. Column chromatography was carried out on Merck Kieselgel H (type 60, 230 mesh ASTM) using the increased pressure supplied by a Medcalf Hyflo pump. Optical rotations were determined using a Perkin-Elmer 141 polarimeter. Light petroleum refers to the fraction of b.p. 60—80 °C. Organic solutions were dried over dried magnesium sulphate and the solvents removed by evaporation under reduced pressure on a rotary evaporator. Yields quoted are based on the pure material.

Clavulanic Acid (**1**).—Benzyl clavulanate (**17**) (100 mg) in ethanol (5 ml) was hydrogenated over 10% palladium on carbon (30 mg) for 45 min at ambient temperature and 1 atm of hydrogen. The mixture was filtered and the catalyst washed with ethanol; the filtrate and washings were combined and the solvent removed to give clavulanic acid (**1**) as a colourless viscous oil (58 mg, 84%); $\nu_{max.}(CHCl_3)$ 1 795, 1 725, and 1 690 cm^{-1}; δ(C_5D_5N) 3.05 (1 H, d, *J* 18 Hz, 6β-H), 3.60 (1 H, dd, *J* 18 and 2.5 Hz, 6α-H), 4.75 (2 H, d, *J* 7.5 Hz, 9-H_2), 5.58 (1 H, t, *J* 7.5 Hz, 8-H), 5.66 (1 H, s, 3-H), and 6.0 (1 H, d, *J* 2.5 Hz, 5-H). This product decomposed slowly on standing.

General Procedure for the Preparation of Clavulanic Acid Esters.— Method A. A solution of sodium clavulanate (**12**) (19.8 mg) in *N*,*N*-dimethylformamide (0.5 ml) was treated with methyl iodide (0.25 ml) and the solution was stirred at room temperature for 1.5 h. The solvent was removed and the residue subjected to preparative t.l.c. (R_F 0.38; red colour with triphenyltetrazolium chloride spray) eluting with ethyl acetate to give *methyl clavulanate* (**13**) as a colourless oil (10.5 mg, 48%), $[\alpha]_D^{22}$ +38° (*c* 1.0, MeOH); $\nu_{max.}$(film) 1 800, 1 750, and 1 695 cm^{-1}; δ_H 2.49 (1 H, br s, exchanged with D_2O, OH), 3.05 (1 H, dd, *J* 17.5 and 0.8 Hz, 6β-H), 3.54 (1 H, dd, *J* 17.5 and 2.8 Hz, 6α-H), 3.84 (3 H, s, CO_2CH_3), 4.24 (2 H, dd, *J* 7.2 and 6.5 Hz, 9-H_2), 4.93 (1 H, ddd, *J* 7.2, 6.5, and 1.2 Hz, 8-H), 5.07 (1 H, br d, *J* 1.2 Hz, 3-H), and 5.72 (1 H, dd, *J* 2.8 and 0.8 Hz, 5-H); δ_C 46 (t, C-6), 53 (q, OCH_3), 57 (t, C-9), 60 (d, C-3), 87 (d, C-5), 100 (d, C-8), 152 (s, C-2), 167 (s, C-10), and 174 p.p.m. (s, C-7) (Found: C, 50.5; H, 5.45; N, 6.3%; M^+, 213.0635. $C_9H_{11}NO_5$ requires C, 50.7; H, 5.2; N, 6.55%; *M*, 213.0637).

Method B. An ethereal solution of diazomethane was added in excess to clavulanic acid (130 mg) in ethanol (10 ml). After a few minutes at room temperature the solution was evaporated and chromatographed, eluting with ethyl acetate, to give methyl clavulanate (**13**) as a colourless oil (104 mg, 75%) (identical i.r. and n.m.r. with the compound described above).

Method C. Clavulanic acid (**1**) (200 mg) in acetonitrile (5 ml) was stirred and cooled in an ice-bath while methanol (0.5 ml) and dicyclohexylcarbodi-imide (206 mg) were added. The mixture was stirred overnight at room temperature, filtered, and the filtrate evaporated; the resulting residue was chromatographed, using ethyl acetate as eluant; thus *methyl clavulanate* (**13**) was obtained as a clear oil (140 mg, 65%). The compound was identical with the product obtained by methods A and B.

Subsequently, we found that methyl clavulanate crystallised with time and was obtained as colourless needles from methyl acetate–ether–light petroleum, m.p. 62—63 °C.

Using essentially the same procedure described in method A above, isolating by conventional work-up and column chromatography (except where otherwise stated), the following esters were obtained.

p-Nitrobenzyl clavulanate (**14**): eluant, ethyl acetate–cyclohexane (1:1), 60% yield, as colourless needles (chloroform–ether), m.p. 117.5—118 °C, $[\alpha]_D^{20}$ +57.6° (*c* 1.07; $CHCl_3$); $\nu_{max.}$(KBr) 1 783, 1 737, and 1 685 cm^{-1}; δ_H 1.58 (1 H, br s, exchanged with D_2O, OH), 3.05 (1 H, d, *J* 17 Hz, 6β-H), 3.49 (1 H, dd, *J* 17 and 3 Hz, 6α-H), 4.22 (2 H, br d, *J* 7.5 Hz, 9-CH_2), 4.89 (1 H, br t, *J* 7.5 Hz, 8-H), 5.1 (1 H, br s, 3-H), 5.27 (2 H, s, CH_2Ar), 5.68 (1 H, br d, *J* 3 Hz, 5-H), 7.49 (2 H, d, *J* 9 Hz, Ar), and 8.22 (2 H, d, *J* 9 Hz, Ar) (Found: C, 54.05; H, 4.35; N, 8.45%; M^+, 334.0789. $C_{15}H_{14}N_2O_7$ requires C, 53.9; H, 4.2; N, 8.4%; *M*, 334.0801).

p-Bromobenzyl clavulanate (**15**): eluant, ethyl acetate–n-hexane (3:1), 60% yield, as colourless needles (dichloromethane–carbon tetrachloride), m.p. 103—104 °C, $[\alpha]_D^{20}$ +57.8° (*c* 1.0; $CHCl_3$); $\nu_{max.}$($CHCl_3$) 1 800, 1 745, and 1 690 cm^{-1}; δ_H 1.76 (1 H, br s, exchanged with D_2O, OH), 3.12 (1 H, d, *J* 18 Hz, 6β-H), 3.62 (1 H, dd, *J* 18 and 2.5 Hz, 6α-H), 4.31 (2 H, d, *J* 7 Hz, 9-CH_2), 4.97 (1 H, dt, *J* 7 and 1.5 Hz, 8-H), 5.18 (1 H, d, *J* 1.5 Hz, 3-H), 5.27 (2 H, s, CH_2Ar), 5.78 (1 H, d, *J* 2.5 Hz, 5-H), 7.33 (2 H, d, *J* 9 Hz, Ar), and 7.67 (2 H, d, *J* 9 Hz, Ar) (Found: C, 49.1; H, 3.7; Br, 21.8; N, 3.75%; M^+, 367.0052. $C_{15}H_{14}BrNO_5$ requires C, 48.95; H, 3.85; Br, 21.7; N, 3.8%; *M*, 367.0055).

p-Methoxybenzyl clavulanate (**16**): Sephadex LH20 and eluant, chloroform–cyclohexane (1:1), 56.5% yield, as colourless prisms (crystallised from ethyl acetate at −20 °C), m.p. 43—44.5 °C, $[\alpha]_D^{20}$ +62.3° (*c* 1.0; $CHCl_3$); $\nu_{max.}$($CHCl_3$) 3 600, 1 800, 1 743, and 1 694 cm^{-1}; δ_H 1.56 (1 H, br s, exchangeable with D_2O), 3.01 (1 H, d, *J* 17.5 Hz, 6α-H), 3.45 (1 H, dd, *J* 17.5 and 3 Hz, 6β-H), 3.78 (3 H, s, OCH_3), 4.17 (2 H, d, *J* 7 Hz, 9-H_2), 4.83 (1 H, dt, *J* 7 and 1.5 Hz, 8-H), 5.02 (1 H, br s, 3-H), 5.11 (2 H, s, CH_2Ar), 5.64 (1 H, br d, *J* 3 Hz, 5-H), 6.86 (2 H, d, *J* 9 Hz, Ar), and 7.27 (2 H, d, *J* 9 Hz, Ar) (Found: C, 60.3; H, 5.65; N, 4.3%; M^+, 319.1063. $C_{16}H_{17}NO_6$ requires C, 60.12; H, 5.35; N, 4.4%; *M*, 319.1056).

Benzyl clavulanate (**17**): eluant, ethyl acetate–cyclohexane (1:1), 69% yield, as a colourless oil, $[\alpha]_D^{20}$ +47° (*c* 1.21; MeOH); $\nu_{max.}$(film) 1 800, 1 745, and 1 695 cm^{-1}; δ_H 2.25 (1 H, s, exchanged with D_2O, OH), 3.05 (1 H, d, *J* 17 Hz, 6β-H), 3.51 (1 H, dd, *J* 17 and 2.5 Hz, 6α-H), 4.24 (2 H, d, *J* 7.5 Hz, 9-H_2) (1 H, dt, *J* 7.5 and 1.5 Hz, 8-H), 5.15 (1 H, d, *J* 1.5 Hz, 3-H), 5.24 (2 H, s, CH_2Ar), 5.71 (1 H, d, *J* 2.5 Hz, 5-H), and 7.45 (5 H, s, Ar)

(Found: C, 62.15; H, 5.3; N, 4.65%; M^+, 289.0942. $C_{15}H_{15}NO_5$ requires C, 62.3; H, 5.25; N, 4.85%; M, 289.0950).

Phenacyl clavulanate (**18**): eluant, ethyl acetate–cyclohexane (1:1), 55.5% yield, as colourless needles (ethyl acetate), m.p. 89—90 °C, $[\alpha]_D^{20}$ +32.6° (*c* 1.0; MeOH); $\nu_{max.}$(Nujol) 3 530, 1 798, 1 742, and 1 700 cm^{-1}; δ_H 1.82 (1 H, s, exchanged with D_2O, OH), 3.06 (1 H, dd, *J* 17 and 1 Hz, 6β-H), 3.55 (1 H, dd, *J* 17 and 3 Hz, 6α-H), 4.2 (2 H, d, *J* 8 Hz, 9-CH_2), 5.0 (1 H, dt, *J* 8 and 1 Hz, 8-H), 5.23 (1 H, s, 3-H), 5.58 (2 H, s, CH_2COPh), 5.74 (1 H, dd, *J* 3 and 1 Hz, 5-H), and 7.7 (5 H, m, Ar) (Found: C, 60.45; H, 4.65; N, 4.45%; M^+, 317.089 44. $C_{16}H_{15}NO_6$ requires C, 60.55; H, 4.75; N, 4.4%; M, 317.089 93).

n-Nonyl clavulanate (**19**): eluant, ethyl acetate–n-hexane (3:1 grading to 2:1), 25% yield, as a colourless oil; $\nu_{max.}$(film) 1 800, 1 745, and 1 690 cm^{-1}; δ_H 0.95 (3 H, m, CH_3), 1.1—2.0 [14 H, m, $(CH_2)_7CH_3$], 3.12 (1 H, d, *J* 17.5 Hz, 6β-H), 3.60 (1 H, dd, *J* 17.5 and 3 Hz, 6α-H), 4.1—4.5 (4 H, m, 9-H_2 and CO_2CH_2), 5.05 (1 H, dt, *J* 8 and 1.5 Hz, 8-H), 5.18 (1 H, s, 3-H), and 5.82 (1 H, dd, *J* 3 and 1.5 Hz, 5-H) (Found: M^+, 325.1890. $C_{17}H_{27}NO_5$ requires M, 325.1889).

Pivaloyloxymethyl clavulanate (**20**): not subjected to chromatography, 90% yield, as a pale yellow oil; $\nu_{max.}$(film) 1 800 and 1 760 cm^{-1}; δ_H 1.26 [9 H, s, $C(CH_3)_3$], 3.13 (1 H, d, *J* 17 Hz, 6β-H), 3.62 (1 H, dd, *J* 17 and 2.5 Hz, 6α-H), 4.3 (2 H, d, *J* 7.5 Hz, 9-H_2), 5.0 (1 H, dt, *J* 7.5 and 1.5 Hz, 8-H), 5.16 (1 H, d, *J* 1.5 Hz, 3-H), 5.79 (1 H, d, *J* 2.5 Hz, 5-H), and 5.92 (2 H, s, CO_2CH_2).

Phthalidyl clavulanate (**21**). Reaction between sodium clavulanate and 3-bromophthalide gave a diastereoisomeric mixture of the phthalidyl esters. Separation of the diastereoisomers was achieved using high-pressure liquid chromatography (40 cm × 10 mm column of silica gel, Merckosorb SI 60, 5 μm; elution with ethyl acetate at a flow rate of 3 ml/min). The first eluted ester (retention time 7.15 min) (10% yield) was obtained as colourless needles (ethyl acetate), m.p. 102 °C; $\nu_{max.}$(Nujol) 1 790 and 1 755 cm^{-1}; δ_H[$(CD_3)_2CO$] 3.14 (1 H, d, *J* 17 Hz, 6β-H), 3.76 (1 H, dd, *J* 17.5 and 2.5 Hz, 6α-H), 4.25 (2 H, d, *J* 7.5 Hz, 9-H_2), 5.0 (1 H, dt, *J* 7.5 and 1.5 Hz, 8-H), 5.4 (1 H, d, *J* 1.5 Hz, 3-H), 5.82 (1 H, d, *J* 2.5 Hz, 5-H), 7.7 (1 H, s, CO_2CH), and 8.06 (4 H, m, Ar) (Found: M^+, 331.0696. $C_{16}H_{13}NO_4$ requires M, 331.0692). Continued elution gave the second diastereoisomer (R_t 8.85 min), 10% yield, as a colourless oil; $\nu_{max.}$(CH_2Cl_2) 1 800 and 1 780 cm^{-1}; δ_H 2.42 (1 H, br s, exchanged with D_2O, OH), 3.12 (1 H, d, *J* 18 Hz, 6β-H), 3.60 (1 H, dd, *J* 18 and 2.5 Hz, 6α-H), 4.30 (2 H, d, *J* 7.5 Hz, 9-H_2), 5.0 (1 H, dt, *J* 7.5 and 1.5 Hz, 8-H), 5.12 (1 H, d, *J* 1.5 Hz, 3-H), 5.76 (1 H, d, *J* 2.5 Hz, 5-H), 7.52 (1 H, s, CO_2CH), and 7.85 (4 H, m, Ar).

Phthalimidomethyl clavulanate (**22**): eluant, ethyl acetate–light petroleum (2:1), 40% yield, as colourless crystals (ethyl acetate–light petroleum), m.p. 163 °C; $\nu_{max.}$($CHCl_3$) 1 805, 1 785, 1 760, and 1 735 cm^{-1}; δ_H 1.7 (1 H, s, OH), 3.08 (1 H, d, *J* 17 Hz, 6β-H), 3.56 (1 H, dd, *J* 17 and 2.5 Hz, 6α-H), 4.25 (2 H, s, *J* 6 Hz, 9-H_2), 4.95 (1 H, dt, *J* 6 and 1 Hz, 8-H), 5.11 (1 H, s, 3-H), 5.75 (1 H, d, *J* 2.5 Hz, 5-H), 5.87 (2 H, s, CH_2N), and 7.75—8.28 (4 H, m, Ar).

One other example was prepared by the diazoalkane procedure described by method B.

Benzhydryl clavulanate (**23**). Clavulanic acid (**1**) and diphenyldiazomethane were brought into reaction in tetrahydrofuran at 0 °C followed by overnight reaction at room temperature. The solvent was evaporated and the residue chromatographed, elution with ethyl acetate gave the product as a colourless oil (60% yield), $\nu_{max.}$($CHCl_3$) 3 400, 1 805, 1 755, and 1 700 cm^{-1}; δ_H 2.04 (1 H, s, OH), 3.02 (1 H, d, *J* 17 Hz, 6β-H), 3.52 (1 H, dd, *J* 17 and 2.5 Hz, 6α-H), 4.22 (2 H, d, *J* 7 Hz, 9-H_2), 4.94 (1 H, m, 8-H), 5.27 (1 H, m, 3-H), 5.75 (1 H, d, *J* 2.5 Hz, 5-H), 7.03 (1 H, s, CHAr), and 7.45 (10 H, s, Ar).

The following esters were prepared using the dicyclohexylcarbodi-imide coupling technique described in method C above.

Phenyl clavulanate (**24**): eluant, ethyl acetate–n-hexane (1:1), 59% yield, as a clear oil, $\nu_{max.}$(film) 1 800, 1 770, and 1 690 cm^{-1}; δ_H 2.18 (1 H, br s, OH), 3.06 (1 H, dd, *J* 17 and 0.9 Hz, 6β-H), 3.54 (1 H, dd, *J* 17 and 2.6 Hz, 6α-H), 4.29 (2 H, d, *J* 7.5 Hz, 9-H_2), 5.1 (1 H, dt, *J* 7.5 and 1.5 Hz, 8-H), 5.29 (1 H, d, *J* 1.5 Hz, 3-H), 5.76 (1 H, dd, *J* 2.6 and 0.9 Hz, 5-H), and 7.35 (5 H, m, Ar) (Found: C, 61.75; H, 5.15; N, 5.5%; M^+, 275.0777. $C_{14}H_{13}NO_5$ requires C, 61.1; H, 4.75; N, 5.1%; M, 275.0794).

p-*Tolyl thioclavulanate* (**36**): eluant, ethyl acetate–n-hexane, (1:1, grading to neat ethyl acetate), 17% yield, as colourless crystals (chloroform–light petroleum), m.p. 126—128 °C, $\nu_{max.}$(Nujol) 1 800, 1 715, and 1 700 cm^{-1}; δ_H 1.9 (1 H, br s, OH), 2.45 (3 H, s, CH_3), 3.20 (1 H, d, *J* 17.5 Hz, 6β-H), 3.68 (1 H, dd, *J* 17.5 and 2.5 Hz, 6α-H), 4.37 (2 H, d, *J* 7.5 Hz, 9-H_2), 5.16 (1 H, dt, *J* 7.5 and 1.5 Hz, 8-H), 5.23 (1 H, br d, *J* 1.5 Hz, 3-H), 5.90 (1 H, d, *J* 2.5 Hz, 5-H), and 7.42 (4 H, s, Ar) (Found: M^+, 305.071 83. $C_{15}H_{15}NO_4S$ requires M, 305.072 17).

Pyridylethyl clavulanate (**25**): eluant, ethyl acetate, 50% yield, as a colourless oil, $\nu_{max.}$($CHCl_3$) 3 400, 1 805, 1 755, and 1 700 cm^{-1}; δ_H 2.20 (1 H, br s, OH), 2.97 (1 H, d, *J* 17 Hz, 6β-H), 3.11 (2 H, t, *J* 7 Hz, CH_2Ar), 3.42 (1 H, dd, *J* 17 and 2.5 Hz, 6α-H), 4.15 (2 H, d, *J* 7 Hz, 9-H_2), 4.45—4.9 (3 H, m, CO_2CH_2 and 8-H), 4.97 (1 H, m, 3-H), 5.51 (1 H, d, *J* 2.5 Hz, 5-H), 7.0—7.4 (2 H, m, 3-H and 5-H, Ar), 7.45—7.85 (1 H, m, Ar), and 8.35—8.70 (1 H, m, Ar).

An alternative mixed anhydride procedure was used for the preparation of the following compound.

2,2,2-*Trichloroethyl clavulanate* (**26**). A suspension of sodium clavulanate (221 mg) in dry tetrahydrofuran (5 ml) was stirred and cooled to 0 °C. A solution of trichloroethyl chloroformate (211 mg) in dry tetrahydrofuran (1 ml) was added over 20 min. The mixture was allowed to reach room temperature and stirred overnight. The suspension was filtered and the filtrate concentrated. Chromatography using ethyl acetate–n-hexane (2:1) as eluant gave fractions containing the pure compound (**26**) which was isolated as a colourless oil (22 mg, 6%); $\nu_{max.}$ (film) 1 800, 1 760, and 1 690 cm^{-1}; δ_H 1.56 (1 H, br s, OH), 3.07 (1 H, dd, *J* 17.5 and 0.7 Hz, 6β-H), 3.56 (1 H, dd, *J* 17.5 and 2.5 Hz, 6α-H), 4.24 (2 H, d, *J* 7.5 Hz, 9-H_2), 4.69 (1 H, d, *J* 12 Hz, $CHCCl_3$), 4.92 (1 H, d, *J* 12 Hz, $CHCCl_3$), 5.02 (1 H, dt, *J* 7.5 and 1.3 Hz, 8-H), 5.19 (1 H, d, *J* 1.3 Hz, 3-H), and 5.73 (1 H, dd, *J* 2.5 and 0.7 Hz, 5-H) (Found: M^+, 328.9621. $C_{10}H_{10}Cl_3NO_5$ requires M, 328.9625).

Hydrogenation and Hydrogenolysis of Esters of Clavulanic Acid (**1**).—(a) *Reduction of methyl clavulanate* (**13**). Methyl clavulanate (80 mg) in ethyl acetate (5 ml) was shaken in a hydrogen atmosphere over 10% palladium on carbon (25 mg) for 24 h at ambient temperature and pressure. A further quantity of catalyst (25 mg) was added and the hydrogenation continued for 24 h. Evaporation of the solvent followed by chromatography using ethyl acetate, as eluant, gave *methyl dihydroclavulanate* (**33**) as a 2 : 1 mixture of epimers in the form of a colourless oil (24.6 mg, 30%); $\nu_{max.}$($CHCl_3$) 3 400, 1 790, and 1 740 cm^{-1}; δ_H 1.7—2.4 (2 H, br m, 8-H_2 for two isomers), 2.20 (1 H, br s, exchangeable with D_2O, OH), 2.95 (1 H, d, *J* 17 Hz, 6β-H), 3.47 and 3.50 (1 H, both dd, *J* 17 and 2.5 Hz, 6α-H, for two isomers), 3.88 (3 H, s, CH_3), 4.2—4.9 (4 H, m, 9-H_2, 2-H and 3-H, for two isomers), 5.43 (2/3 H, d, *J* 2.5 Hz, 5-H, major epimer), and 5.66 (1/3 H, br d, *J* 2.5 Hz, 5-H, minor epimer). Partial separation of the epimers was achieved by h.p.l.c. using a 30 cm × 1 mm column of silica gel, Merckosorb SI 60, 5 μm; using ethyl acetate as eluant at a flow rate of 3 ml/min and refractive index detection. The retention of the epimers was 10.5 min (minor epimer) and 11.1 min (major epimer) the latter had the following spectral characteristics; $\nu_{max.}$($CHCl_3$) 3 540, 1 792, and 1 750 cm^{-1}; δ_H 1.7—2.2 (1 H, br, exchanges with D_2O, OH), 2.02 (2 H, m, 8-H_2), 2.87 (1 H, dd, *J* 16 and 1 Hz, 6β-

H), 3.32 (1 H, dd, *J* 16 and 2.5 Hz, 6α-H), 3.73 (3 H, s, CH_3), 3.80 (2 H, m, 9-H_2), 4.2 (1 H, d, *J* 6.5 Hz, 3-H), 4.47 (1 H, m, 2-H), and 5.25 (1 H, dd, *J* 2.5 and 1 Hz, 5-H).

(b) *Hydrogenolysis of benzyl clavulanate* (**17**). (i) A solution of benzyl clavulanate (109 g) in ethanol (1.8 l) and water (300 ml) was shaken with hydrogen over 10% palladium on carbon (15 g), in the presence of sodium hydrogen carbonate (32 g), at ambient temperature and pressure, for 25 min. The suspension was filtered through Celite and the filtrate concentrated to low volume; the residue was redissolved in water and acetone added (*ca.* 10 × volume). The product was filtered off, washed with acetone, and air-dried to give *sodium clavulanate* (**12**) as the crystalline tetrahydrate (76.4 g, 69%). Recrystallisation from aqueous acetone gave the analytically pure compound as feathery needles, m.p. 68 °C; $[\alpha]_D^{20}$ +47.1° (*c* 1; H_2O), $\nu_{max.}$ (KBr) 3 100—3 650vs, 1 792, 1 688, 1 662, and 1 595 cm^{-1}; δ_H 3.08 (1 H, d, *J* 17.5 Hz, 6β-H), 3.61 (1 H, dd, *J* 17.5 and 3 Hz, 6α-H), 4.19 (2 H, d, *J* 8 Hz, 9-H_2), the signal for 8-H was obscured by HOD peak at δ 4.66, 4.96 (1 H, s, 3-H), and 5.73 (1 H, br d, *J* 3 Hz, 5-H) [Found: C, 32.65; H, 4.4; N, 4.7; Na, 8.2, H_2O (Karl Fischer) 24.3%. $C_8H_8NNaO_5 \cdot 4H_2O$ requires C, 32.75; H, 5.5; N, 4.8; Na, 7.85; H_2O 24.5%].*

(ii) A mixture of benzyl clavulanate (94 mg) and sodium hydrogen carbonate (28 mg) in ethanol was hydrogenated over 10% palladium on carbon (30 mg) at ambient temperature for 60 min. The catalyst was filtered off and washed with water; the filtrate and washings were combined and evaporated. Residual water was azeotroped with acetone by repeated addition and evaporation. The resultant white solid was dissolved in *N,N*-dimethylformamide (2.5 ml) followed by the addition of *p*-bromobenzyl bromide (245 mg) and the mixture left at room temperature for 2 h. Chromatography and gradient elution, starting with ethyl acetate–n-hexane (1:1) grading to neat ethyl acetate, gave two products. The less-polar compound (**28**) crystallised from methylene chloride–carbon tetrachloride as thin colourless rods (11 mg, 9%), m.p. 134—134.5 °C; $[\alpha]_D^{25}$ +23° (*c* 1.16; MeOH); $\nu_{max.}$($CHCl_3$) 1 800, 1 745, and 1 690 cm^{-1}; δ_H 1.7 (1 H, br s, exchangeable with D_2O, OH), 3.02 (1 H, d, *J* 17 Hz, 6β-H), 3.53 (1 H, dd, *J* 17 and 2.5 Hz, 6α-H), 4.10 (2 H, d, *J* 7.5 Hz, 9-H_2), 5.16 (2 H, s, CH_2Ar), 5.35 (1 H, s, 3-H), 5.16 (1 H, d, *J* 2.5 Hz, 5-H), 7.22 (2 H, d, *J* 8.5 Hz, Ar), and 7.53 (2 H, d, *J* 8.5 Hz, Ar). The olefinic proton (8-H) was obscured by the ester methylene protons and the C-3 proton; however, a decoupling experiment indicated that irradiation at δ 5.3 caused the doublet at δ 4.10 (9-H_2) to collapse to a singlet (Found: M^+, 367.0055. $C_{15}H_{14}{}^{79}BrNO_5$ requires *M*, 367.0055). The data indicated that this compound was p-*bromobenzyl isoclavulanate* (*E*-isomer) (**28**) its stereostructure was confirmed by *X*-ray crystallographic analysis. Continued elution gave the more-polar compound (**15**) which crystallised from methylene chloride–carbon tetrachloride as needles (52 mg, 43%), m.p. 103—104 °C. This product had i.r. and n.m.r. spectra identical with those of *p*-bromobenzyl clavulanate (*Z*-isomer) (**15**) previously described. The structure was confirmed and the absolute stereochemistry was elucidated by *X*-ray analysis.

(iii) Benzyl clavulanate (650 mg) in ethyl acetate (20 ml) was hydrogenated at room temperature and 1 atm over platinum oxide (650 mg) for 2 h. When 2 mol equiv. of hydrogen had been absorbed the suspension was filtered through Celite and the filtrate treated with a solution of diazotoluene in ether at 0 °C. Evaporation of the solvent and chromatography using gradient elution with ethyl acetate–light petroleum mixtures gave *benzyl dihydroclavulanate* (**34**) as a 1:1 mixture of epimers (82 mg, 13%). Repeated chromatography eluting with the same solvent afforded separation of the less-polar epimer, $[\alpha]_D^{22}$ +70° (*c* 0.77; MeOH); $\nu_{max.}$($CHCl_3$) 3 400, 1 790, and 1 740 cm^{-1}; δ_H 1.70 (1 H, br s, exchangeable with D_2O, OH), 2.09 (2 H, br t, *J* 6 Hz, 8-H_2), 2.92 (1 H, d, *J* 17 Hz, 6β-H), 3.42 (1 H, dd, *J* 17 and 2.5 Hz, 6α-H), 3.78 (2 H, br t, *J* 6 Hz, 9-H_2), 4.20—4.80 (2 H, m, 2-H and 3-H), 5.28 (2 H, s, CH_2Ar), 5.39 (1 H, d, *J* 2.5 Hz, 5-H), and 7.49 (5 H, s, Ar). The remaining fractions contained both epimers, $[\alpha]_D^{22}$ +67.6° (*c* 0.79; MeOH); $\nu_{max.}$($CHCl_3$) 3 400, 1 790, and 1 740 cm^{-1}; δ_H 1.70 (1 H, br s, exchangeable with D_2O, OH), 1.74 and 2.09 (2 H, both t, *J* 6 Hz, 8-H_2 for both epimers), 2.92 (1 H, br d, *J* 17 Hz, 6β-H), 3.40 and 3.35 (1 H, two dd, *J* 17 and 2.5 Hz, 6α-H, for both epimers), 3.75 (2 H, two br t, *J* 6 Hz, 9-H_2 for both epimers), 4.2—4.9 (2 H, m, 2-H and 3-H), 5.28 (2 H, s, CH_2Ar), 5.39 (1/2 H, d, *J* 2.5 Hz, 5-H least polar epimer), and 5.62 (1/2 H, dd, *J* 2.5 and 1 Hz, 5-H more polar epimer) [Found: M^+ 263.1153. $C_{14}H_{17}NO_4$ (loss of CO) requires *M*, 263.1157].

(iv) Hydrogenolysis of benzyl clavulanate (2.45 g) in tetrahydrofuran (150 ml) over 10% palladium on carbon (2.45 g) for 2.5 h at ambient temperature and 1 atm followed by neutralisation with sodium hydrogen carbonate (712 mg) gave after conventional work-up and chromatography, using n-butanol–ethanol–water (4:1:1) as eluant, *sodium deoxyclavulanate* (**39**) as a white solid (780 mg, 45%), $\nu_{max.}$(KBr) 1 780, 1 700, and 1 600 cm^{-1}; δ_H 1.52 (3 H, dd, *J* 7 and 1.5 Hz, CH_3), 2.98 (1 H, d, *J* 18 Hz, 6β-H), 3.53 (1 H, dd, *J* 18 and 2.5 Hz, 6α-H), and 5.65 (1 H, d, *J* 2.5 Hz, 5-H); the remainder of the protons were obscured by the HOD peak at δ 5.3. The gross structure of this compound was confirmed by treating the salt (10 mg) with *p*-bromobenzyl bromide (50 mg) in dry *N,N*-dimethylformamide (0.5 ml) at room temperature for 2 h. Work-up followed by chromatography with ethyl acetate–n-hexane (1:4) gave p-*bromobenzyl deoxyclavulanate* (**40**) as a colourless oil (12 mg, 70%); δ_H 1.62 (3 H, dd, *J* 7 and 2 Hz, CH_3), 2.96 (1 H, dd, *J* 17.3 and 1 Hz, 6β-H), 3.47 (1 H, dd, *J* 17.3 and 2.6 Hz, 6α-H), 4.58 (1 H, dq, *J* 7 and 1.7 Hz, 8-H), 5.0 (1 H, m, 3-H), 5.13 (2 H, s, CH_2Ar), 5.63 (1 H, dd, *J* 2.6 and 1 Hz, 5-H), 7.18 (2 H, d, *J* 8.3 Hz, Ar), and 7.50 (2 H, d, *J* 8.3 Hz, Ar). The absolute structure was confirmed by *X*-ray crystallographic analysis of the *p*-toluamide (**41**) which was prepared as follows. A solution of deoxyclavulanic acid (**42**) in tetrahydrofuran (*ca.* 183 mg obtained from the hydrogenolysis of benzyl clavulanate in tetrahydrofuran over an equal weight of 10% palladium-on-carbon) was cooled to 0 °C and *p*-toluidine (107 mg) and dicyclohexylcarbodi-imide (206 mg) were added with stirring. The mixture was stirred at room temperature for 4 h, filtered, and the filtrate evaporated. The residual solid was purified by chromatography with ethyl acetate–cyclohexane (1:1) as the eluant to yield N-p-*tolyldeoxyclavulanamide* (**41**) as a crystalline solid (44 mg, 50%) which was further purified by recrystallisation from methylene chloride–n-hexane to yield colourless plates, m.p. 153—154 °C; $\nu_{max.}$($CHCl_3$) 3 350, 1 805, 1 705, and 1 690 cm^{-1}; δ_H 1.65 (3 H, dd, *J* 7 and 1.6 Hz, 9-CH_3), 2.30 (3 H, s, $ArCH_3$), 3.07 (1 H, d, *J* 17 Hz, 6β-H), 3.48 (1 H, dd, *J* 17 and 2.5 Hz, 6α-H), 4.78—5.10 (2 H, m, 8-H and 3-H), 5.60 (1 H, d, *J* 2.5 Hz, 5-H), 7.10 (2 H, d, *J* 9 Hz, Ar), 7.39 (2 H, d, *J* 9 Hz, Ar), and 8.21 (1 H, s, NH).

(v) A mixture of benzyl clavulanate (289 mg) and sodium hydrogen carbonate (84 mg) dissolved in 50% aqueous ethanol (10 ml) was shaken under 1 atm of hydrogen over 10% palladium on carbon (85 mg) for 3 days at ambient temperature. The suspension was filtered though Kieselguhr and the filtrate evaporated. To the residual solid was added sequentially *N,N*-dimethylformamide (5 ml), potassium carbonate (140 mg), and a five-molar excess of methyl iodide (700 mg). After overnight reaction the products were extracted into chloroform and this extract was washed well with water, dried,

* In the Perkin-Elmer Model 240 Elemental Analyser a stream of dry helium is passed over the sample before combustion; since this removes loosely bound water, the results for hydrogen analysis for the tetrahydrate are lower than theoretical.

and concentrated to an oil. Chromatography of this with ethyl acetate–light petroleum mixtures as eluant gave, in order of elution, *methyl deoxyclavulanate* (**43**) as an colourless oil (6 mg, 8%), $[\alpha]_D^{20}$ +66.4° (*c* 1.31; MeOH); $\nu_{max.}$($CHCl_3$) 1 800, 1 745, and 1 700 cm^{-1}; δ_H 1.68 (3 H, dd, *J* 7 and 2 Hz, 9-H_3), 3.05 (1 H, d, *J* 17 Hz, 6β-H), 3.59 (1 H, dd, *J* 17 and 3 Hz, 6α-H), 3.84 (3 H, s, CO_2CH_3), 4.72 (1 H, dq, *J* 7 and 2 Hz, 8-H), 5.10 (1 H, m, 3-H), and 5.77 (1 H, d, *J* 3 Hz, 5-H) (Found: C, 54.65; H, 5.8; N, 6.9%; M^+, 197.0685. $C_9H_{11}NO_4$ requires C, 54.8; H, 5.6; N, 7.1%; *M*, 197.0688). The second product eluted was impure and in the form of an oil in low yield (2%) but was thought to contain predominantly methyl dihydrodeoxyclavulanate (t.l.c.). *Methyl isoclavulanate* (**27**) was isolated as a colourless oil (18.5 mg, 21%), $[\alpha]_D^{22}$ +38.4° (*c* 1.77; MeOH); $\nu_{max.}$($CHCl_3$) 1 800, 1 745, and 1 690 cm^{-1}; δ_H 2.0 (1 H, br s, exchanged with D_2O, OH), 3.09 (1 H, d, *J* 17 Hz, 6β-H), 3.60 (1 H, dd, *J* 17 and 2.5 Hz, 6α-H), 3.87 (3 H, s, CO_2CH_3), 4.21 (2 H, br d, *J* 7.5 Hz, 9-H_2), 5.40 (1 H, br t, *J* 7.5 Hz, 8-H), 5.42 (1 H, s, 3-H), and 5.75 (1 H, d, *J* 2.5 Hz, 5-H) (Found: M^+, 213.0633. $C_9H_{11}NO_5$ requires *M*, 213.0637).

Methyl clavulanate (**13**) was isolated as a colourless oil (42.5 mg, 48%) with i.r. and n.m.r. spectra identical with those of the pure, analysed sample above. *Methyl dihydroclavulanate* (**33**) as an epimeric mixture (*ca.* 1:1) was isolated as a colourless oil (18 mg, 21%); $\nu_{max.}$($CHCl_3$) 3 400, 1 790, and 1 740 cm^{-1}; δ_H 1.90 (1 H, br s, exchanged with D_2O, OH), 1.80—2.40 (2 H, br t, *J* 6 Hz, 8-H_2 for both isomers), 2.84 (1 H, d, *J* 17 Hz, 6β-H), 3.36 and 3.40 (1 H, both dd, *J* 17 and 2.5 Hz, 6α-H), 3.72 (2 H, br s, CO_2CH_3), 4.20—4.90 (4 H, br m, 9-H_2, 3-H and 2-H, for both epimers), and 5.32 and 5.54 (1 H, both d, *J* 2.5 Hz, 5-H for both epimers).

Photolytic Isomerisation of Benzyl Clavulanate (**17**).—A solution of benzyl clavulanate (300 mg) in dry benzene (400 ml) was irradiated for 5 h under nitrogen in a quartz vessel enclosed in a Hanovia Photochemical 'Reading Reactor' which contains two low-pressure lamps of 45 W, each with an energy maximum at 254 nm. The solvent was removed and the resulting yellow oil was fractionated on silica gel; elution with cyclohexane–ethyl acetate (1:2) gave, in order of elution *benzyl isoclavulanate* (*E*-isomer) (**29**) as a colourless oil (120 mg, 40%) and benzyl clavulanate (*Z*-isomer) as a colourless oil (150 mg, 50%). The *E*-isomer had $[\alpha]_D^{25}$ +29.3° (*c* 2.62; MeOH); $\nu_{max.}$(CH_2Cl_2) 3 550, 1 795, 1 740, and 1 685 cm^{-1}; δ_H 1.85 (1 H, s, exchanges with D_2O, OH), 2.98 (1 H, dd, *J* 17 and 1 Hz, 6β-H), 3.45 (1 H, dd, *J* 17 and 2.5 Hz, 6α-H), 4.05 (2 H, d, *J* 7 Hz, 9-H_2), 5.18 (2 H, s, CH_2Ar), 5.32 (1 H, s, 3-H), 5.35 (1 H, m, obscured by signal at δ 5.32, 8-H), 5.63 (1 H, dd, *J* 2.5 and 1 Hz, 5-H), and 7.36 (5 H, s, Ar) (Found: C, 62.55; H, 5.4; N, 4.6%; M^+, 289.0949. $C_{15}H_{15}NO_5$ requires C, 62.3; H, 5.25; N, 4.85%; *M*, 289.0950). The *Z*-isomer was identified by comparison of its i.r. and n.m.r. spectra with the corresponding spectra of authentic material.

Deprotection of Benzyl Isoclavulanate (**29**) *by Hydrogenolysis*.—A mixture of benzyl isoclavulanate (60 mg) and sodium hydrogen carbonate (17.6 mg) in ethanol was hydrogenated over 10% palladium on carbon (20 mg) at 20 °C and 1 atm pressure for 1.75 h, after which time t.l.c. (ethyl acetate–cyclohexane, 1:1) showed the reaction to be complete. The suspension was filtered and the catalyst washed with water; the filtrate and washings were combined and evaporated. The residue was twice treated with ethanol and evaporated, once with acetone and evaporated, and finally triturated with acetone and diethyl ether to give *sodium isoclavulanate* (**30**) as an off-white powder (30 mg, 66%); $\nu_{max.}$(KBr) 3 400s, 1 780, 1 680, and 1 615 cm^{-1}; δ_H 3.10 (1 H, d, *J* 17.5 Hz, 6β-H), 3.64 (1 H, dd, *J* 17.5 and 3 Hz, 6α-H), 4.18 (2 H, d, *J* 7.5 Hz, 9-H_2), 5.22 (2 H, m, 8-H and 3-H), and 5.86 (1 H, d, *J* 3 Hz, 5-H).

Photolytic Isomerisation of Phenacyl Clavulanate (**18**).—Repetition of the above photolysis experiment but with phenacyl clavulanate (100 mg) and an irradiation time of 3 h gave, after solvent removal and column chromatography with ethyl acetate–cyclohexane (1:1) as eluant, two components; the more-polar of these was unchanged starting material (60 mg, 60%). The less-polar component was examined by h.p.l.c. and found to be a mixture of two compounds which were separated by preparative h.p.l.c. on Reeve-Angel microparticulate silica gel; 7 μm, using ethyl acetate as eluant at a flow rate of 3 ml/min and detection by u.v. absorbance at 278 nm. The first eluted compound (R_t 5.8 min) was the oxetane (1S,2aS,5aR,8aR,9aR)-*tetrahydro-1-hydroxymethyl*)-2a-*phenyl*-1H,7H-*azeto*[2,1-b]-*oxeto*[3′,2′:4,5]*pyrano*[3,4-d]*oxazole*-5,7(5aH)-*dione* (**45**) which crystallised from ethyl acetate–light petroleum as colourless prisms (20 mg, 20%), m.p. 188 °C; $\nu_{max.}$($CHCl_3$) 3 550, 1 800, and 1 770 cm^{-1}; δ_H 3.32 (2 H, m, 8-H_2), 3.88 (2 H, br d, *J* 9 Hz, CH_2OH), 4.56 and 5.18 (2 H, ABq, *J* 12 Hz, 3-H_2), 5.17 (1 H, t, *J* 9 Hz, 1-H), 5.38 (1 H, m, 8a-H), 5.83 (1 H, s, 5a-H), and 3.42 (5 H, s, Ar); δ_C 46.2 (t, C-8), 59.0 (t, CH_2OH), 61.6 (d, C-5a), 72.0 (t, C-3), 84.1 (s, C-2a or C-9a), 86.9 (2 × d, C-8a and C-1), 88.3 (s, C-2a or C-9a), 125.1 (d, C-3′,3′), 128.0 (d, C-2′,2′ and C-4′), 137.2 (s, C-1′), 166.7 (s, C-5), and 175.4 (s, C-7) (Found: C, 60.35; H, 4.9; N, 4.2%; M^+, 317.089 69. $C_{16}H_{15}NO_6$ requires C, 60.55; H, 4.75; N, 4.4%; *M*, 317.089 93). The structure of this tetracyclic β-lactam (**45**) was confirmed by *X*-ray crystallographic analysis. Subsequent elution of the column afforded the geometric isomer, *phenacyl isoclavulanate* (**31**) (R_t 6.4 min) which was obtained as a colourless oil (15 mg, 15%); $\nu_{max.}$(film) 3 480, 1 790, 1 750, and 1 690 cm^{-1}; δ_H 3.02 (1 H, dd, *J* 17 and 1 Hz, 6β-H), 3.52 (1 H, dd, *J* 17 and 3 Hz, 6α-H), 4.27 (2 H, d, *J* 9 Hz, 9-H_2), 5.43 (1 H, s, 3-H), 5.48 (1 H, m, 8-H), 5.52 (2 H, s, CH_2COPh), 5.73 (1 H, dd, *J* 3 and 1 Hz, 5-H), and 7.7 (5 H, m, Ar) (Found: M^+, 317.089 82. $C_{16}H_{15}NO_6$ requires *M*, 317.089 93).

General Procedure for Hydrolysis of Hydrolysable Esters of Clavulanic Acid (**1**): *Hydrolysis of Methyl Clavulanate* (**13**).—In a typical experiment methyl clavulanate (2.21 g) was dissolved in water (100 ml) and to the stirred solution, cooled to 0 °C, was added dropwise 0.05M-sodium hydroxide (150 ml) during 70 min. The orange solution was evaporated to a gum which was redissolved in water (10 ml) and to this solution acetone was added gradually until an orange coloured oil began to separate. The supernatant liquid was decanted and diluted with acetone to *ca.* 300 ml. Crystalline material separated upon standing; this was collected by filtration, washed on the filter with acetone, and then air-dried. This material was further purified by recrystallisation from aqueous acetone, using pre-washed activated charcoal to remove coloured material, to give sodium clavulanate (**12**) isolated as the crystalline tetrahydrate (2.13 g, 70%).

Hydrolysis of Methyl Clavulanate (**13**) *under Conditions of Controlled* pH.—In a typical experiment a solution of methyl clavulanate (0.5 g) in water (10 ml) at 20 °C was maintained at pH 9.5 by the addition of 1.0M-sodium hydroxide from an automatic titrator. The reaction was allowed to proceed for 1.5 h and then worked up in a similar manner to the preceding experiment to yield sodium clavulanate (**12**) isolated as the crystalline tetrahydrate (0.41 g, 60%). The half-life of methyl clavulanate was *ca.* 15 min. The material produced in both hydrolysis experiments proved to be identical (m.p., i.r., and h.p.l.c.) with an authentic sample of the tetrahydrate.

Amides of Clavulanic Acid (**1**).—(*a*) Solutions of sodium clavulanate (150 mg) in water (1 ml), *p*-toluidine hydrochloride (80 mg) in water (1 ml), and 1-cyclohexyl-3-(2-morpholinoethyl)-

N-methylcarbodi-imidinium toluene-*p*-sulphonate (210 mg) in dioxane–water (1:2) (3 ml) were mixed at 0 °C with stirring. After 1 h, the precipitated toluamide was collected by filtration, washed with water, and dried *in vacuo* to yield N-p-*tolylclavulanamide* (**37**) as colourless crystals (95 mg, 65%) which crystallised from ethanol as needles, m.p. 226 °C; $\nu_{max.}$(Nujol) 3 330, 1 800, 1 680, and 1 615 cm^{-1}; δ_H[$(CD_3)_2SO$] 2.23 (3 H, s, CH_3), 3.09 (1 H, d, *J* 17 Hz, 6β-H), 3.63 (1 H, dd, *J* 17 and 3 Hz, 6α-H), 4.0 (2 H, m, collapses to d with D_2O, 9-H_2), 4.57 (1 H, t, *J* 5 Hz, exchanges with D_2O, OH), 4.77 (1 H, t, *J* 7 Hz, 8-H), 5.14 (1 H, s, 3-H), 5.74 (1 H, d, *J* 3 Hz, 5-H), 7.12 (2 H, d, *J* 8 Hz, Ar), 7.45 (2 H, d, *J* 8 Hz, Ar), and 10.2 (1 H, br s, exchanges with D_2O, NH) (Found: C, 62.25; H, 5.6; N, 9.65. $C_{15}H_{16}N_2O_4$ requires C, 62.5; H, 5.6; N, 9.7%).

(*b*) A 0.3M-solution of clavulanic acid (**1**) in tetrahydrofuran was prepared by hydrogenolysis of benzyl clavulanate (**17**) (2.89 g) in dry redistilled tetrahydrofuran (30 ml) over 10% palladium on carbon (0.8 g) for 20 min at 22 °C and 1 atm of hydrogen. The catalyst was filtered off and an aliquot of the filtrate was titrated to pH 7 with 1.0M-sodium hydroxide solution to confirm that it had the desired concentration. The remainder of the filtrate (14 ml) and solutions of benzylamine (0.4 g) in ethyl acetate (100 ml) and dicyclohexylcarbodi-imide (0.8 g) in dichloromethane (100 ml) were mixed quickly together. The mixture was evaporated to near dryness and dichloromethane (100 ml) added. After 1 h at room temperature the suspension was cooled to 2—3 °C, and the filtrate evaporated. The residue was triturated with ethyl acetate–ether and the amide was collected by filtration and dried. Crystallisation from ethyl acetate gave N-*benzylclavulanamide* (**38**) as colourless crystals (0.8 g, 67%), m.p. 139 °C; $\nu_{max.}$(Nujol) 3 300, 1 800, 1 698, and 1 660 cm^{-1}; δ_H[$(CD_3)_2SO$] 3.01 (1 H, d, *J* 17 Hz, 6β-H), 3.56 (1 H, dd, *J* 17 and 3 Hz, 6α-H), 4.0 (2 H, m, collapses to d with D_2O), 4.27 (2 H, d, *J* 6 Hz, collapses to s with D_2O), 4.57 (1 H, t, *J* 5 Hz, exchanges with D_2O, OH), 4.72 (1 H, t, *J* 7 Hz, 8-H), 5.0 (1 H, s, 3-H), 5.77 (1 H, d, *J* 3 Hz, 5-H), 7.36 (5 H, s, Ar), 8.75 (1 H, br t, *J* 6 Hz, exchanges with D_2O) (Found: C, 62.6; H, 5.7; N, 9.9. $C_{15}H_{16}N_2O_4$ requires C, 62.5; H, 5.5; N, 9.7%).

General Procedures for the Acylation of Esters of Clavulanic Acid (**1**)

(A) *With Carboxylic Acids.—Method* 1. Phenoxyacetic acid (152 mg) was added to a cold (ice-bath) solution of benzyl clavulanate (**17**) (289 mg) in dry dichloromethane (5 ml) followed by the portionwise addition of dicyclohexylcarbodi-imide (230 mg). The mixture was stirred overnight at room temperature after which the reaction was complete as judged by t.l.c. (ethyl acetate–cyclohexane, 1:1); the mixture was filtered and the filtrate washed with water, dried, and concentrated. The residual oil was chromatographed using gradient elution with ethyl acetate–light petroleum (1:8) grading to neat ethyl acetate to give *benzyl* 9-O-*phenoxyacetylclavulanate* (**51**) as a colourless oil (156 mg, 37%); $[\alpha]_D^{20}$ +20.3° (*c* 1.06; MeOH); $\nu_{max.}$ ($CHCl_3$) 1 810, 1 740—1 770, and 1 705 cm^{-1}; δ_H 3.01 (1 H, *J* 17 Hz, 6β-H), 3.44 (1 H, dd, *J* 17 and 3 Hz, 6α-H), 4.54 (2 H, s, 9-H_2), 4.76 (3 H, m, CH_2OPh and 8-H), 5.05 (1 H, s, 3-H), 5.14 (2 H, s, CH_2Ar), 5.64 (1 H, d, *J* 3 Hz, 5-H), and 6.78—7.32 (10 H, m, Ar) (Found: C, 65.65; H, 5.3; N, 3.1%. $C_{23}H_{21}NO_7$ requires C, 65.25; H, 4.95; N, 3.3%). By a similar procedure the following compounds were prepared.

Benzyl 9-O-*phenylacetylclavulanate* (**52**): eluant, ethyl acetate–light petroleum (1:1), 35% yield, as a colourless oil; $\nu_{max.}$ ($CHCl_3$) 1 805 and 1 740 cm^{-1}; δ_H 3.10 (1 H, d, *J* 17 and 1 Hz, 6β-H), 3.61 (1 H, dd, *J* 17 and 2.5 Hz, 6α-H), 3.70 (2 H, s, $OCOCH_2Ph$), 4.79 (3 H, m, 8-H and 9-H_2), 5.17 (1 H, s, 3-H), 5.20 (2 H, s, OCH_2Ph), 5.78 (1 H, d, *J* 2.5 and 1 Hz, 5-H), 7.44 (5 H, s, Ar), and 7.50 (5 H, s, Ar) (Found: M^+, 407.1395. $C_{23}H_{21}NO_6$ requires *M*, 407.1369).

Benzyl 9-O-(α-*benzyloxycarbonyl*)*phenylacetylclavulanate* (**53**): eluant, ethyl acetate–light petroleum (1:8 grading to neat ethyl acetate), 73% yield, as a colourless viscous oil; $[\alpha]_D^{22}$ +33° (*c* 0.57; MeOH); $\nu_{max.}$($CHCl_3$) 1 800 and 1 735 cm^{-1}; δ_H 3.08 (1 H, d, *J* 17 Hz, 6β-H), 3.55 (1 H, dd, *J* 17 and 2.5 Hz, 6α-H), 4.79 (1 H, s, CHPh), 4.82 (3 H, m, 8-H and 9-H_2), 5.13 (1 H, s, 3-H), 5.27 (4 H, s, 2 × OCH_2Ph), 5.75 (1 H, d, *J* 2.5 Hz, 5-H), and 7.50 (15 H, s, Ar).

Benzyl 9-O-(α-*phenoxycarbonyl*)*phenylacetylclavulanate* (**54**): eluant, ethyl acetate–light petroleum (1:6), 79% yield, as a colourless gum; $[\alpha]_D^{22}$ +13° (*c* 0.54; MeOH); $\nu_{max.}$($CHCl_3$) 1 800 and 1 735 cm^{-1}; δ_H 3.03 (1 H, d, *J* 17 Hz, 6β-H), 3.53 (1 H, dd, *J* 17 and 2.5 Hz, 6α-H), 4.9 (4 H, m, 8-H, 9-H_2, and CHPh), 5.15 (1 H, s, 3-H), 5.23 (2 H, s, CH_2Ph), 5.75 (1 H, d, *J* 2.5 Hz, 5-H), and 7.0—7.7 (15 H, m, Ar).

Phthalimidomethyl 9-O-(α-*benzyloxycarbonyl*)*phenylacetylclavulanate* (**55**): eluant, ethyl acetate–light petroleum (1:1), 64% yield as a gum; $\nu_{max.}$($CHCl_3$) 1 805, 1 790, and 1 740 cm^{-1}; δ_H 3.0 (1 H, d, *J* 17 Hz, 6β-H), 3.52 (1 H, dd, *J* 17 and 2.5 Hz, 6α-H), 4.72 (1 H, s, CHPh), 4.78 (3 H, m, 8-H and 9-H_2), 5.08 (1 H, s, 3-H), 5.25 (2 H, s, CO_2CH_2Ph), 5.71 (1 H, d, *J* 2.5 Hz, 5-H), 5.82 (2 H, s, CH_2N), 7.0—7.43 (10 H, m, Ar), and 7.65—8.20 (4 H, m, Ar).

Method 2. Essentially the same procedure as described for the preparation of the preceding compounds was followed except that 1 mol equiv. of pyridine was included and acetone replaced dichloromethane as solvent. By this method the following compounds were obtained.

Benzyl 9-O-*phenoxyacetylclavulanate* (**51**): eluant, ethyl acetate–light petroleum (1:8 grading to neat ethyl acetate), 58% yield, as a colourless oil; all spectroscopic properties fully agree with those previously described.

Benzyl 9-O-*acetylclavulanate* (**56**): eluant, ethyl acetate–n-hexane (1:1), 23% yield, as a gum; $\nu_{max.}$($CHCl_3$) 1 800 and 1 735br cm^{-1}; δ_H 2.08 (3 H, s, CH_3), 3.13 (1 H, d, *J* 17 Hz, 6β-H), 3.60 (1 H, dd, *J* 17 and 2.5 Hz, 6α-H), 4.55—5.0 (3 H, m, 8-H and 9-H_2), 5.28 (1 H, s, 3-H), 5.28 (2 H, s, CH_2Ar), 5.80 (1 H, d, *J* 2.5 Hz, 5-H), and 7.47 (5 H, s, Ar).

(B) *With Acid Chlorides.*—A stirred solution of benzyl clavulanate (2.51 g) in dichloromethane (30 ml) at −30 °C was treated with pyridine (775 μl) directly followed by the dropwise addition of chloroacetyl chloride (690 μl) in dichloromethane (10 ml) during 10 min. After 10 min, t.l.c. (ethyl acetate–light petroleum, 1:1) indicated complete reaction. The mixture was poured into aqueous hydrochloric acid and the aqueous solution extracted with dichloromethane. The organic phase was washed successively with dilute hydrochloric acid, aqueous sodium hydrogen carbonate, water, and saturated brine and then dried and evaporated to give *benzyl* 9-O-*chloroacetylclavulanate* (**57**) as a pale yellow oil (3.1 g, 97%) which was homogeneous by t.l.c. (ethyl acetate–light petroleum, 1:1). After repeat chromatography using the same solvent the product was obtained as a colourless oil which had, $[\alpha]_D^{20}$ +42° (*c* 1.2; $CHCl_3$); $\nu_{max.}$($CHCl_3$) 1 804, 1 755br, and 1 700 cm^{-1}; δ_H 2.98 (1 H, d, *J* 17 Hz, 6β-H), 3.42 (1 H, dd, *J* 17 and 3 Hz, 6α-H), 3.98 (2 H, s, CH_2Cl), 4.60—4.98 (3 H, m, 8-H and 9-H_2), 5.09 (1 H, s, 3-H), 5.15 (2 H, s, CH_2Ar), 5.67 (1 H, d, *J* 3 Hz, 5-H), and 7.32 (5 H, s, Ar) (Found: C, 56.1; H, 4.4; Cl, 9.65; N, 3.6. $C_{17}H_{16}ClNO_6$ requires C, 55.8; H, 4.35; Cl, 9.7; N, 3.85%). Using the same process the following compound was prepared.

Benzyl 9-O-*dichloroacetylclavulanate* (**58**): eluant, ethyl acetate–cyclohexane (1:3), 98% yield, as a colourless oil, $[\alpha]_D^{20}$ +39.6° (*c* 1.0; $CHCl_3$); $\nu_{max.}$($CHCl_3$) 1 808, 1 755, and 1 697 cm^{-1}; δ_H 3.05 (1 H, d, *J* 17 Hz, 6β-H), 3.50 (1 H, dd, *J* 17 and 3 Hz, 6α-H), 4.82 (3 H, s, 8-H and 9-H_2), 5.10 (1 H, s, 3-H), 5.17 (2 H, s, CH_2Ar), 5.70 (1 H, d, *J* 3 Hz, 5-H), 5.90 (1 H, s, $CHCl_2$), and 7.32 (5 H, s, Ar) (Found: C, 51.0; H, 3.95; Cl, 17.55;

N, 3.4%; M^+, 399.0268. $C_{17}H_{15}Cl_2NO_6$ requires C, 51.0; H, 3.8; Cl, 17.7; N, 3.5%; M, 399.0275).

(C) *Mixed Anhydride.*—Isobutyl chloroformate (68.5 mg) was added dropwise to a stirred mixture of phenoxyacetic acid (76 mg), *N*-methylmorpholine (50.5 mg), and dichloromethane (5 ml) at < -5 °C. Stirring was continued at *ca.* -5 °C for 15 min, benzyl clavulanate (144.5 mg) in dichloromethane (5 ml) was then added, and the resulting mixture was allowed to reach ambient temperature. After 3 days the reaction mixture was worked up in the usual manner and chromatography with ethyl acetate–light petroleum (1:1) as eluant afforded a pure sample of *benzyl* 9-O-*phenoxyacetylclavulanate* (**51**) as a colourless oil (92.5 mg, 44%), which had spectral properties identical with those of an authentic sample. Further elution of the column gave recovered benzyl clavulanate (**17**) (72.8 mg, 50%).

(D) *With Isocyanates.*—(i) Benzyl clavulanate (0.4 g) and trimethylsilyl isocyanate (1 ml) were mixed in the minimum amount of dichloromethane to give one phase. The solution was set aside at room temperature for 3 days. The reaction mixture was then decanted from a small amount of insoluble material and poured into vigorously stirred aqueous phosphate buffer (pH 6.8). The product mixture was extracted with ethyl acetate and the extract washed with water and dried (Na_2SO_4). Evaporation of the solvent left a pale brown gum; t.l.c. showed that hydrolysis was incomplete therefore the gum was redissolved in aqueous tetrahydrofuran and allowed to stand at room temperature. The pure product was isolated by preparative h.p.l.c. on a 40 cm × 10 mm column of silica gel, Merckosorb SI 60, 5 μm. Elution with ethyl acetate at a flow rate of 3 ml/min and detection by u.v. absorbance at 278 nm, gave *benzyl* 9-O-*carbamoylclavulanate* (**59**) as an oil (10% yield), $\nu_{max.}$(film) 3 490, 3 380, 1 800, and 1 700—1 760 cm^{-1} (complex broad band); δ_H 1.64 (2 H, br, exchanges with D_2O, NH_2), 3.08 (1 H, d, *J* 17 Hz, 6β-H), 3.49 (1 H, dd, *J* 17 and 3 Hz, 6α-H), 4.5—5.0 (3 H, m, 8-H and 9-H_2), 5.12 (1 H, s, 3-H), 5.22 (2 H, s, CH_2Ar), 5.73 (1 H, d, *J* 3 Hz, 5-H), and 7.39 (5 H, s, Ar).

(ii) A solution of benzyl clavulanate (**17**) (289 mg) in methyl isocyanate (3 ml) was allowed to stand at room temperature for 48 h. Evaporation of the excess of isocyanate yielded *benzyl* 9-O-(N-*methylcarbamoyl*)*clavulanate* (**60**) as a pale yellow oil in quantitative yield; $\nu_{max.}$(film) 3 400, 1 808, 1 755, 1 730, 1 715, and 1 700 cm^{-1}; δ_H 2.79 (3 H, m, CH_3), 3.04 (1 H, dd, *J* 18 and 1.5 Hz, 6β-H), 3.54 (1 H, dd, *J* 18 and 3 Hz, 6α-H), 4.5—5.0 (3 H, m, 8-H and 9-H_2), 5.11 (1 H, s, 3-H), 5.22 (2 H, s, CH_2Ar), 5.72 (1 H, dd, *J* 3 and 1.5 Hz, 5-H), and 7.4 (5 H, s, Ar).

(iii) Benzyl clavulanate (**17**) (1.4 g) and phenyl isocyanate (1.7 g) were mixed and the mixture set aside at room temperature for 5 days. The crude mixture was subjected to column chromatography using ethyl acetate–light petroleum (1:3) as eluant to give *benzyl* 9-O-(N-*phenylcarbamoyl*)*clavulanate* (**61**) isolated as a solid (0.4 g, 20%), which melted at room temperature (*ca.* 22 °C); $\nu_{max.}$(film) 3 330, 1 802, and 1 735br cm^{-1}; δ_H 3.03 (1 H, d, *J* 17 Hz, 6β-H), 3.5 (1 H, dd, *J* 17 and 3 Hz, 6α-H), 4.6—5.0 (3 H, m, 8-H and 9-H_2), 5.09 (1 H, s, 3-H), 5.18 (2 H, s, CH_2Ph), 5.71 (1 H, br d, *J* 3 Hz, 5-H), 6.62 (1 H, br, NH), and 7.0—7.4 (10 H, m, Ar).

(iv) To a stirred solution of benzyl clavulanate (**17**) (2.89 g) in dry dichloromethane (20 ml) was added benzoyl isocyanate (2.5 g). The mixture was evaporated and ethyl acetate–cyclohexane (1:1) (10 ml) was added to the residue; solid material was filtered off and the filtrate was subjected to column chromatography using ethyl acetate–cyclohexane (1:1) as eluant. Fractions containing the desired product were combined and cooled and the deposited benzamide was filtered off and discarded. The filtrate was evaporated and dichloromethane (3—4 ml) added; more benzamide was precipitated and this was filtered off and the filtrate added to isopropyl alcohol (40 ml). On cooling, the product crystallised slowly and was collected by filtration, washed quickly with ether, and dried to give *benzyl* 9-O-(N-*benzoylcarbamoyl*)*clavulanate* (**62**) as a crystalline solid (1.1 g, 25%), m.p. 80 °C; $\nu_{max.}$(Nujol) 3 260, 1 805, 1 760, 1 750, and 1 695 cm^{-1}; δ_H 3.04 (1 H, d, *J* 17 Hz, 6β-H), 3.53 (1 H, dd, *J* 17 and 3 Hz, 6α-H), 4.7—5.0 (3 H, m, 8-H and 9-H_2), 5.1 (1 H, s, 3-H), 5.21 (2 H, s, CH_2Ph), 5.73 (1 H, d, *J* 3 Hz, 5-H), 7.3—8.0 (10 H, m, Ar), and 8.28 (1 H, br s, exchanges with D_2O, NH).

(v) To benzyl clavulanate (**17**) (6.3 g) in dry redistilled tetrahydrofuran at 5 °C was added 1M-trichloroacetyl isocyanate in dichloromethane (22.8 ml). After only 2 min, t.l.c. (ethyl acetate–cyclohexane, 1:1) showed absence of starting material. The product was isolated by evaporation of the solvent to give a quantitative yield (10.7 g) of *benzyl* 9-O-(N-*trichloroacetylcarbamoyl*)*clavulanate* (**63**) as a colourless oil which had $\nu_{max.}$(film) 3 600br, 1 810, 1 750, and 1 700sh cm^{-1}; δ_H 3.04 (1 H, d, *J* 17 Hz, 6β-H), 3.48 (1 H, dd, *J* 17 and 3 Hz, 6α-H), 4.84 (3 H, m, 8-H and 9-H_2), 5.06 (1 H, s, 3-H), 5.15 (2 H, s, CH_2Ph), 5.67 (1 H, d, *J* 3 Hz, 5-H), 7.28 (5 H, s, Ar), and 8.49 (1 H, s, exchanges with D_2O, NH).

Deprotection of Acyl Derivatives of Clavulanic Acid Esters by Hydrogenolysis.—(i) *Deprotection of* (**51**). A mixture containing benzyl 9-*O*-phenoxyacetylclavulanate (**51**) (141 mg) and sodium hydrogen carbonate (56 mg) in ethanol (5 ml) and ethyl acetate (1 ml) was hydrogenated at ambient temperature and 1 atm of hydrogen, for 20 min, over 10% palladium on carbon (47 mg). The suspension was filtered through Celite and the filter pad washed with water; the aqueous filtrate was evaporated. The ^1H n.m.r. spectrum of the residual solid showed it to be a mixture of sodium deoxyclavulanate (**39**) and sodium phenoxyacetate in a 1:1.2 ratio, and not the required sodium salt of the acyl clavam (**64**). A mixture of salts (340 mg; obtained from this experiment and other repeat experiments) was dissolved in *N*,*N*-dimethylformamide (2 ml) and benzyl bromide (700 mg) was added; the solution was then stirred for 3.5 h at ambient temperature. The solution was diluted with ethyl acetate, washed with water, dried, and concentrated. Chromatography using ethyl acetate–light petroleum (1:10) gave a pure sample of *benzyl deoxyclavulanate* (**44**) as a colourless oil (84 mg, 37%), $[\alpha]_D^{22}$ +59.7° (*c* 0.66; MeOH); $\nu_{max.}$($CHCl_3$) 1 800, 1 740, and 1 702 cm^{-1}; δ_H 1.61 (3 H, dd, *J* 7 and 1.5 Hz, CH_3), 2.97 (1 H, dd, *J* 17.5 and 1.5 Hz, 6β-H), 3.47 (1 H, dd, *J* 17.5 and 3 Hz, 6α-H), 4.6 (1 H, dq, *J* 7 and 1.5 Hz, 8-H), 5.01 (1 H, m, 3-H), 5.17 (2 H, s, CH_2Ar), 5.65 (1 H, dd, *J* 3 and 1.5 Hz, 5-H), and 7.35 (5 H, s, Ar) (Found: M^+, 273.1007. $C_{15}H_{15}NO_4$ requires M, 273.1001).

(ii) *Deprotection of* (**51**). Benzyl 9-*O*-phenoxyacetylclavulanate (**51**) (198 mg) in dry tetrahydrofuran (15 ml) was hydrogenolysed over 10% palladium on carbon (66 mg) at ambient temperature and pressure. After 15 min the suspension was filtered through Kieselgel and the filtrate divided into three equal portions. The first portion was evaporated to dryness to give 9-O-*phenoxyacetylclavulanic acid* (**64**) as an oil (50 mg, 96%); $\nu_{max.}$($CHCl_3$) 2 300—3 400, 1 800, 1 730—1 750, and 1 695 cm^{-1}; δ_H 3.01 (1 H, d, *J* 17 Hz, 6β-H), 3.44 (1 H, dd, *J* 17 and 2.5 Hz, 6α-H), 4.58 (2 H, s, CH_2OPh), 4.83 (3 H, m, 8-H and 9-H_2), 5.05 (1 H, s, 3-H), 5.69 (1 H, d, *J* 2.5 Hz, 5-H), 6.8—7.4 (5 H, m, Ar), and 11.08 (1 H, br s, OH). The second portion was treated with an excess of ethereal diazomethane at 0 °C; evaporation of the ether followed by column chromatography with ethyl acetate–cyclohexane (1:1) as eluant afforded a pure sample of *methyl* 9-O-*phenoxyacetylclavulanate* (**65**) as a colourless oil (25 mg, 46%); $[\alpha]_D^{21}$ +17.4° (*c* 0.78; MeOH); $\nu_{max.}$($CHCl_3$) 1 805, 1 735—1 755, and 1 700 cm^{-1}; δ_H 3.08 (1 H, d, *J* 17 Hz, 6β-H), 3.62 (1 H, dd, *J* 17 and 2.5 Hz, 6α-H), 3.88

(3 H, s, CH_3), 4.71 (2 H, s, CH_2OPh), 4.93 (3 H, m, 8-H and 9-H_2), 5.18 (1 H, s, 3-H), 5.82 (1 H, d, *J* 2.5 Hz, 5-H), and 6.9—7.6 (5 H, m, Ar). Finally, the third portion was neutralised with sodium hydrogen carbonate (12 mg) in water (1 ml) and the solvents removed to give *sodium* 9-O-*phenoxyacetylclavulanate* (**66**) as a white solid (54.5 mg, 99%); $\nu_{max.}$(KBr) 1 780, 1 750, 1 690, and 1 600—1 610 cm^{-1}.

(iii) *Deprotection of* (**60**). This compound also readily eliminated the carbamoyl side-chain on attempted debenzylation by hydrogenolysis in ethanol. Benzyl 9-*O*-(*N*-methylcarbamoyl)clavulanate (150 mg) in ethanol (7 ml) was hydrogenated over 10% palladium on carbon (150 mg) under normal reaction conditions. Customary work-up gave a product which was identified from its ^1H n.m.r spectrum as methylammonium deoxyclavulanate. Short-column ion-exchange on Amberlite IR 120 cation exchange resin (Na^+ form) eluting with water afforded sodium deoxyclavulanate (**39**) as a white solid (25 mg, 28%).

(iv) *Deprotection of* (**60**). Benzyl 9-*O*-(*N*-methylcarbamoyl)-clavulanate (175 mg) in freshly distilled tetrahydrofuran (5 ml) was similarly hydrogenated over 10% palladium on carbon (50 mg). When uptake of hydrogen had almost ceased (*ca.* 10 min) the catalyst was removed by filtration, washed with tetrahydrofuran, and the filtrate and washings combined and evaporated to yield 9-O-(N-*methylcarbamoyl)clavulanic acid* (**67**) as a nearly colourless oil (120 mg, 93%) which was homogeneous by t.l.c.; $\nu_{max.}$(film) 3 400br, 2 600, 1 810, 1 720br, and 1 700br cm^{-1}; δ_H 2.75 (3 H, br d, CH_3), 3.06 (1 H, d, *J* 16 Hz, 6β-H), 3.49 (1 H, dd, *J* 16 and 3 Hz, 6α-H), 4.6—5.1 (4 H, m, 8-H, 9-H_2, and 3-H), 5.71 (1 H, d, *J* 3 Hz, 5-H), and 8.95 (1 H, br s, NH). To a solution of this acid (100 mg) in ethanol (5 ml) and water (1 ml) was added solid sodium hydrogen carbonate (300 mg). After effervescence had ceased the excess of base was filtered off and the solvent evaporated to yield *sodium* 9-O-(N-*methylcarbamoyl)clavulanate* (**68**) as a buff-coloured solid (87 mg, 80%); $\nu_{max.}$(DMSO solution liquid film) 1 790, 1 700, and 1 630 cm^{-1}; δ_H 2.88 (3 H, s, CH_3), 3.2 (1 H, d, *J* 18 Hz, 6β-H), 3.7 (1 H, dd, *J* 18 and 3 Hz, 6α-H), and 5.7 (1 H, d, *J* 3 Hz, 5-H); the remaining protons were obscured by the water peak. The n.m.r. solution showed signs of rapid degradation and insoluble material was deposited with time.

The following compounds were prepared by the same debenzylation procedure described for compound (**60**); in each example a portion of the product obtained was converted into the corresponding methyl ester to substantiate further the structure of the deprotected acyl derivative.

9-O-*Phenylacetylclavulanic acid* (**69**) as an oil (91% yield); $\nu_{max.}$($CHCl_3$) 1 802, 1 740br, and 1 700 cm^{-1}; δ_H 3.01 (1 H, d, *J* 17.5 Hz, 6β-H), 3.43 (1 H, dd, *J* 17.5 and 2.5 Hz, 6α-H), 3.59 (2 H, s, CH_2Ph), 4.6—4.95 (3 H, m, 8-H and 9-H_2), 5.04 (1 H, s, 3-H), 5.66 (1 H, d, *J* 2.5 Hz, 5-H), 7.28 (5 H, s, Ar), and 8.75 (1 H, br s, OH). *Methyl* 9-O-*phenylacetylclavulanate* (**70**) as a clear oil (78% yield); $\nu_{max.}$($CHCl_3$) 1 803, 1 750, and 1 700 cm^{-1}; δ_H 3.09 (1 H, d, *J* 17 Hz, 6β-H), 3.60 (1 H, dd, *J* 17 and 2.5 Hz, 6α-H), 3.7 (2 H, s, CH_2Ph), 3.83 (3 H, s, CH_3), 4.65—5.1 (3 H, m, 8-H and 9-H_2), 5.13 (1 H, s, 3-H), 5.88 (1 H, d, *J* 2.5 Hz, 5-H), and 7.4 (5 H, s, Ar). 9-O-[*Carboxy(phenyl)acetyl*]*clavulanic acid* (**71**) as a colourless oil (90% yield); $\nu_{max.}$($CHCl_3$) 1 805, 1 700—1 740br, and 1 690 cm^{-1}; δ_H 2.97 (1 H, d, *J* 17 Hz, 6β-H), 3.41 (1 H, dd, *J* 17 and 2.5 Hz, 6α-H), 4.5—5.0 (4 H, m, 8-H, 9-H_2, and CHPh), 5.02 (1 H, s, 3-H), 5.64 (1 H, d, *J* 2.5 Hz, 5-H), 7.33 (5 H, s, Ar), and 10.62 (2 H, br s, 2 × OH). *Methyl* 9-O-[*methoxycarbonyl(phenyl)acetyl*]*clavulanate* (**72**) as a colourless oil (72% yield); $\nu_{max.}$($CHCl_3$) 1 805, 1 750, 1 735, and 1 700 cm^{-1}; δ_H 3.02 (1 H, d, *J* 17 Hz, 6β-H), 3.47 (1 H, dd, *J* 17 and 3 Hz, 6α-H), 3.72 (3 H, s, CO_2CH_3), 3.76 (3 H, s, CO_2CH_3), 4.6—4.9 (4 H, m, 8-H, 9-H_2, and CHPh), 5.04 (1 H, s, 3-H), 5.68 (1 H, d, *J* 3 Hz, 5-H), and 7.37 (5 H, s, Ar).

Direct Acetylation of Sodium Clavulanate (**12**) *with Acetic Anhydride*.—Solid sodium clavulanate tetrahydrate (250 mg) was added to cold (0—5 °C) acetic anhydride (5 ml) and the mixture was stirred at this temperature overnight. After removal of the solvent the crude product was submitted to column chromatography. Elution with n-butanol–ethanol–water (16:4:7) afforded *sodium* 9-O-*acetylclavulanate* (**73**) as a white amorphous solid (55 mg, 25%); $\nu_{max.}$(KBr) 1 780, 1 725, 1 695, and 1 610 cm^{-1}; δ_H 2.02 (3 H, s, CH_3), 3.06 (1 H, d, *J* 17 Hz, 6β-H), 3.53 (1 H, dd, *J* 17 and 2.5 Hz, 6α-H), 4.87 (2 H, m, 3-H and 8-H), and 5.67 (1 H, d, *J* 2.5 Hz, 5-H); the signal for 9-H_2 was obscured by the HOD peak at δ 4.6.

Trimethylammonium Salt of Benzyl 9-O-*Hydroxysulphonyl-clavulanate* (**46**).—Benzyl clavulanate (**17**) (57.8 mg) dissolved in dry *N*,*N*-dimethylformamide (0.8 ml) was treated with trimethylamine–sulphur trioxide complex (55.6 mg) and set aside at ambient temperature for 18 h. The solvent was removed and the residue dissolved in chloroform; this solution was evaporated and the residue extracted several times with diethyl ether to remove unchanged benzyl clavulanate. The ether-insoluble material was identified spectroscopically as the sulphate (**46**) and was obtained as a solid (61 mg, 69%); $\nu_{max.}$($CHCl_3$) 2 700, 2 500w, 2 450, 2 350w, 1 800, 1 745, and 1 695 cm^{-1}; δ_H 3.0 (9 H, s, $H\overset{+}{N}Me_3$), 3.65 (1 H, dd, *J* 17 and 2.5 Hz, 6α-H; the other doublet of the ABX system for the 6β-H is obscured by the trimethylammonium signal), 4.71 (2 H, d, *J* 8 Hz, 9-H_2), 4.8—5.5 (4 H, 3-H, 8-H, and CH_2Ar), 5.82 (1 H, d, *J* 2.5 Hz, 5-H), and 7.52 (5 H, s, Ar).

Oxidation of the Esters of Clavulanic Acid (**1**).—*Method A*. Benzyl clavulanate (**17**) (0.75 g) in dichloromethane (10 ml) was added to a suspension of pyridinium chlorochromate (1.0 g) in dichloromethane (20 ml) with stirring. After 20 min the mixture was diluted with diethyl ether (50 ml) and then filtered through Celite and the filtrate collected. The solvent was removed and the resulting residue was chromatographed rapidly on silica gel using ethyl acetate–light petroleum (1:1) elution, to give a 1:1.2 mixture of the *E*- and *Z*-isomers of *benzyl* (2R,5R)-3-*oxoethylidene*-7-*oxo*-4-*oxa*-1-*azabicyclo*[3.2.0]*heptane*-2-*carboxylate* (**47**) as a gum (79 mg, 10%); $\nu_{max.}$($CHCl_3$) 1 810, 1 750, 1 675, and 1 650 cm^{-1}; δ_H (*E*-isomer) 3.11 (1 H, d, *J* 17 Hz, 6β-H), 3.55 (1 H, dd, *J* 17 and 3 Hz, 6α-H), 5.15 (2 H, s, CH_2Ar), 5.65 (1 H, d, *J* 1.5 Hz, 2-H), 5.82 (1 H, dd, *J* 5 and 1.5 Hz, 8-H), 5.86 (1 H, d, *J* 3 Hz, 5-H), 7.28 (5 H, s, Ar), and 9.52 (1 H, d, *J* 5 Hz, CHO); (*Z*-isomer) 3.18 (1 H, d, *J* 17 Hz, 6β-H), 3.59 (1 H, dd, *J* 17 and 3 Hz, 6α-H), 5.15 (2 H, s, CH_2Ar), 5.24 (1 H, d, *J* 1 Hz, 2-H), 5.33 (1 H, dd, *J* 7.5 and 1 Hz, 8-H), 5.76 (1 H, d, *J* 3 Hz, 5-H), 7.28 (5 H, s, Ar), and 9.81 (1 H, d, *J* 7.5 Hz, CHO) (Found: M^+, 287.079 108. $C_{15}H_{13}NO_5$ requires *M*, 287.079 363).

In a similar reaction between methyl clavulanate (**13**) (0.95 g) and pyridinium chlorochromate (3 g), conventional work-up and chromatography gave a 1.5:1 (*E*:*Z*) mixture of the geometric isomers of *methyl* (2R,5R)-3-*oxoethylidene*-7-*oxo*-4-*oxa*-1-*azabicyclo*[3.2.0]*heptane*-2-*carboxylate* (**48**) as a gum (48 mg, 5%); $\nu_{max.}$($CHCl_3$) 1 815, 1 760, 1 680, and 1 650 cm^{-1}; δ_H (*E*-isomer) 3.13 (1 H, d, *J* 17 Hz, 6β-H), 3.58 (1 H, dd, *J* 17 and 3 Hz, 6α-H), 3.77 (3 H, s, CH_3), 5.63 (1 H, d, *J* 1.5 Hz, 2-H), 5.86 (1 H, dd, *J* 5 and 1.5 Hz, 8-H), 5.90 (1 H, d, *J* 3 Hz, 5-H), and 9.55 (1 H, d, *J* 5 Hz, CHO); (*Z*-isomer) 3.21 (1 H, d, *J* 17 Hz, 6β-H), 3.63 (1 H, dd, *J* 17 and 3 Hz, 6α-H), 3.77 (3 H, s, CH_3), 5.24 (1 H, d, *J* 1 Hz, 2-H), 5.38 (1 H, dd, *J* 7.5 and 1 Hz, 8-H), 5.79 (1 H, d, *J* 3 Hz, 5-H), and 9.83 (1 H, d, *J* 7.5 Hz, CHO).

The following oxidation was carried out using a slightly modified procedure. To a stirred solution of *p*-methoxybenzyl clavulanate (**16**) (300 mg) in dry dichloromethane (20 ml) was added pyridinium chlorochromate (405 mg) and powdered sodium acetate (30 mg) and the whole was stirred for a further

1.5 h. The mixture was worked up as before and fast column chromatography (benzene–ethyl acetate, 7:3) afforded p-*methoxybenzyl* (2R,5R)-3-*oxoethylidene*-7-*oxo*-4-*oxa*-1-*azabicyclo*[3.2.0]*heptane*-2-*carboxylate* (**49**) as a 1.2:1 mixture of its *E*- and *Z*-isomers which was obtained as a gum (26 mg, 9%); $\nu_{max.}$($CHCl_3$) 1 810, 1 750, 1 675, and 1 650 cm^{-1}; δ_H (*E*-isomer) 3.09 (1 H, d, *J* 17 Hz, 6β-H), 3.6 (1 H, m, 6α-H), 3.75 (3 H, s, CH_3), 5.09 (2 H, s, CH_2Ar), 5.61 (1 H, d, *J* 1 Hz, 2-H), 5.8 (1 H, dd, *J* 5.5 and 1 Hz, 8-H), 5.8 (1 H, m, 5-H), 6.8 (2 H, d, *J* 9 Hz, Ar), 7.2 (2 H, d, *J* 9 Hz, Ar), 9.52 (1 H, d, *J* 5.5 Hz, CHO); (*Z*-isomer) similar except 3.17 (1 H, d, *J* 17 Hz, 6α-H), 5.21 (1 H, s, 2-H), 5.32 (1 H, d, *J* 7.5 Hz, 8-H), and 9.81 (1 H, d, *J* 7.5 Hz, CHO); no molecular ion observed in the mass spectrum. A similar experiment was performed but the crude product was chromatographed on cellulose powder (Whatman CC31), with ethyl acetate–light petroleum (1:4) as eluant, to give the aldehyde (**49**) which was obtained in 40% yield but was less pure.

Method B. Benzyl clavulanate (100 mg) was dissolved in dichloromethane (3 ml) and active manganese dioxide (1 g) added. The reaction mixture was left for 2 h and filtered; the solvent was removed from the filtrate and the resultant oily residue was chromatographed (gradient elution with ethyl acetate–n-hexane) to yield the aldehyde (**47**) as a colourless oil (15 mg, 15%).

Similarly, a solution of *p*-methoxybenzyl clavulanate (200 mg) in dry toluene (20 ml) was stirred with activated manganese dioxide (2.4 g). After 30 min the solution was filtered and the filtrate concentrated to afford the aldehyde (**49**) as a 1:2.25 mixture of its *E*- and *Z*-isomers (30 mg, 15%).

Method C. Attempted oxidation of benzyl clavulanate (**17**) *to the aldehyde* (**47**) *using the Pfitzner-Moffat method.* Benzyl clavulanate (0.2 g) was added to dry dimethyl sulphoxide (6 ml) and dry benzene (3 ml) containing dicyclohexylcarbodi-imide (430 mg). Anhydrous orthophosphoric acid (69 mg) in dimethyl sulphoxide (2 ml) was added and the mixture stirred at room temperature for 4 h. After this time t.l.c. (ethyl acetate–cyclohexane, 1:1) showed a faster-moving component which gave a blue fluorescence at 366 nm. The precipitated dicyclohexylurea was filtered off and the filtrate diluted with benzene; the solution was washed with water, dried, and evaporated. Fractionation of the residual oil on silica gel and elution with ethyl acetate–cyclohexane (1:1) provided *benzyl* (5R)-7-*oxo*-3-*vinyl*-4-*oxa*-1-*azabicyclo*[3.2.0]*hept*-2-*ene*-2-*carboxylate* (**50**) as a colourless oil (135 mg, 72%); $[\alpha]_D^{20}$ +4° [*c* 0.6; ethyl acetate–cyclohexane (1:1)]; $\lambda_{max.}$(CH_3CN) 317 nm (ε 7 100); $\nu_{max.}$($CHCl_3$) 1 805, 1 710, and 1 635 cm^{-1}; δ_H[$(CD_3)_2CO$] 3.5 (1 H, dd, *J* 17 and 1.5 Hz, 6β-H), 3.86 (1 H, dd, *J* 17 and 3 Hz, 6α-H), 5.25 (2 H, s, CH_2Ar), 5.62 (1 H, dd, *J* 11 and 2 Hz, CHc:CHa*CH*b), 5.88 (1 H, dd, *J* 17.5 and 2.0 Hz, CHc:C*H*aCHb), 5.98 (1 H, dd, *J* 3 and 1.5 Hz, 5-H), 7.08 (1 H, dd, *J* 17.5 and 11 Hz, C*H*c:CHaCHb), and 7.37 (5 H, m, Ar); δ_C 50.5 (t, C-6), 66.6 (t, C-12), 89.7 (d, C-5), 113.2 (s, C-2), 123.7 (d, C-8), 124.1 (dd, C-9), 128.5 (d, 2′,2′ or 3′,3′), 128.7 (d, 4′), 129.1 (d, 2′,2′ or 3′,3′), 137.1 (s, 1′), 161 (s, C-3 or C-10), 161.4 (s, C-3 or C-10), and 177.1 (s, C-7) (Found: M^+, 271.0830. $C_{15}H_{13}NO_4$ requires *M*, 271.0845). The diene (**50**) polymerises with time but is stable for several weeks if stored in solution in the cold.

Ozonolysis of Clavulanic Acid Esters.—In a typical experiment *p*-bromobenzyl clavulanate (**15**) (1.5 g) in ethyl acetate (100 ml) was stirred at −60 °C while a steady stream of ozone was bubbled through the solution. After 1.5 h, t.l.c. (ethyl acetate–cyclohexane, 1:1) indicated that the reaction was complete; at this point the mixture was saturated with ozone. The ozone was replaced by a steady current of nitrogen and the solution was allowed to warm to room temperature. The solution was washed with water and brine and the solvent removed. The resultant solid was crystallised from dichloromethane–light petroleum to give p-*bromobenzyl* (2S,5R)-3,7-*dioxo*-4-*oxa*-1-*azabicyclo*[3.2.0]*heptane*-2-*carboxylate* (**74**) as colourless needles (0.98 g, 71%), m.p. 105—106 °C; $[\alpha]_D^{20}$ +141° (*c* 1.1, MeOH); $\nu_{max.}$(Nujol) 1 810—1 790 and 1 765 cm^{-1}; δ_H 3.23 (1 H, dd, *J* 17.5 and 1 Hz, 6β-H), 3.63 (1 H, d, *J* 17.5 and 3 Hz, 6α-H), 4.88 (1 H, s, 2-H), 5.16 (2 H, s, CH_2Ar), 5.73 (1 H, dd, *J* 3 and 1 Hz, 5-H), 7.18 (2 H, d, *J* 9 Hz, Ar), and 7.48 (2 H, d, *J* 9 Hz, Ar) (Found: C, 45.85; H, 3.05; Br, 23.6; N, 4.1%; M^+, 338.9743. $C_{13}H_{10}{}^{79}BrNO_5$ requires C, 45.9; H, 2.95; Br, 23.5; N, 4.1%; *M*, 338.9742).

Benzyl clavulanate (**17**) (0.6 g) was ozonised in a manner similar to that described in the preceding example to give, after work-up and chromatography (ethyl acetate–cyclohexane, 1:1), *benzyl* (2S,5R)-3,7-*dioxo*-4-*oxa*-1-*azabicyclo*[3.2.0]*heptane*-2-*carboxylate* (**75**). This crystallised from ethanol as colourless needles (352 mg, 65%), m.p. 57 °C; $[\alpha]_D^{20}$ +129° (*c* 1.31; MeOH); $\nu_{max.}$(Nujol) 1 820, 1 785, and 1 760 cm^{-1}; δ_H 3.22 (1 H, dd, *J* 17 and 1 Hz, 6β-H), 3.68 (1 H, dd, *J* 17 and 3 Hz, 6α-H), 4.92 (1 H, s, 2-H), 5.23 (2 H, s, CH_2Ar), 5.77 (1 H, dd, *J* 3 and 1 Hz, 5-H), and 7.36 (5 H, s, Ar) (Found: M^+, 261.0637. $C_{13}H_{11}NO_5$ requires *M*, 261.0637).

Epoxidation of Esters of Clavulanic Acid (**1**).—*p*-Methoxybenzyl clavulanate (**16**) (200 mg) in dichloromethane (15 ml) was stirred with *m*-chloroperbenzoic acid (162 mg) at −15 °C for 1.4 h. The mixture was diluted with chloroform and the solution was washed with aqueous sodium hydrogen carbonate (2 ×) and water (2 ×) and then dried. The solvent was removed to give a 1.2:1 mixture of p-*methoxybenzyl* (2R,3R,5R,3′S)-3′-*hydroxymethyl*-7-*oxo*{[4]*oxa*[1]*azabicyclo*[3.2.0]*heptane*-3-*spiro*-2′-*oxirane*}-2-*carboxylate* (**77**) *and* its (2R,3S,5R,3′R)-*isomer* (**79**) as a gum (218 mg, 100%); $\nu_{max.}$($CHCl_3$) 1 800 and 1 750 cm^{-1}; δ_H 2.35 (1 H, br s, OH), 2.92 and 3.07 (1 H, each d, *J* 17 Hz, 6α-H for each isomer), 3.38 and 3.44 (1 H, each dd, *J* 17 and 3 Hz, 6β-H for each isomer), 3.32 (1 H, m, 3′-H), 3.73 (3 H, s, CH_3), 3.85 (2 H, m, CH_2OH), 4.53 and 4.77 (1 H, each s, 2-H for major and minor isomer, respectively), 5.52 (1 H, m, 5-H), 6.79 (2 H, d, Ar), and 7.19 (2 H, d, Ar); the mass spectrum did not display a molecular ion. The epoxidation of benzyl clavulanate (**17**) (200 mg) was carried out under the same conditions as above to give a 1.5:1 mixture of *benzyl* (2R,3R,5R,3′S)-3′-(*hydroxymethyl*)-7-*oxo*-{[4]*oxa*[1]*azabicyclo*[3.2.0]*heptane*-3-*spiro*-2′-*oxirane*}-2-*carboxylate* (**78**) and its (2R,3R,5R,3′R) *isomer* (**80**) as a white foam (179 mg, 92%); $\nu_{max.}$($CHCl_3$) 1 800 and 1 750 cm^{-1}; δ_H 2.5 (1 H, br s, exchanges with D_2O, OH), 2.7—3.9 (5 H, m, 3′-H, CH_2OH, 6β-H and 6α-H), 4.46 and 4.69 (1 H, each s, 2-H for major and minor isomer, respectively), 5.08 and 5.18 (2 H, each s, CH_2Ar for minor and major isomer, respectively), 5.6 (1 H, m, 5-H), and 7.3 (5 H, s, Ar); M^+, 305. Chromatography of the product on silica gel resulted in extensive decomposition whilst chromatography on cellulose powder (Whatman CC31) afforded a 65% recovery of the product but without separation of the isomers.

Crystal Structure Determinations.—(a) p-*Nitrobenzyl clavulanate* (**14**): *Crystal data.* $C_{15}H_{14}N_2O_7$, M = 334.3, monoclinic, a = 19.528(6), b = 7.795(3), c = 5.025(1) Å, β = 95.85(3)°, U = 760.9 $Å^3$ (by least-squares refinement of the setting angles for 23 reflections), D_c = 1.46, D_m = 1.44 g cm^{-3}, space group $P2_1$, Z = 2, μ (Mo–K_α) = 1.27 cm^{-1}.

Reflections were counted for θ ≤ 27° (Hilger-Watt Y290 diffractometer, graphite monochromated radiation, λ = 0.710 69 Å), and of the total of 1 783 scanned 1 438 had $I \geq 3\sigma I$ and were used in the refinement. The structure was solved by MULTAN and refined by full-matrix least squares with C,O,N anisotropic and hydrogens in calculated positions

(the alcoholic H was not included). The weighting scheme was $W = 1$ when $F_{obs} \leqslant 9.0$ and $W = 9.0/F_{obs}$ for the stronger reflections. The final agreement factors were $R = 0.055$ and $R_w = 0.069$. The alcoholic oxygen was disordered and occupied two sites to roughly equal extent (refined occupation factors 0.55 and 0.45). The standard variations for bond lengths and angles were in the ranges 0.005—0.011 Å and 0.3—0.5°. Refinement was carried out using the XRAY 72 system.

(b) p-*Bromobenzyl clavulanate* (**15**); *Crystal data.* $C_{15}H_{14}BrNO_5$, $M = 368.2$, monoclinic, $a = 13.155(6)$, $b = 4.763(2)$, $c = 12.670(5)$ Å, $\beta = 95.22(2)°$, $U = 790.6$ Å^3 (by least-squares refinement of the setting angles of 23 reflections), space group $P2_1$, $Z = 2$, μ (Mo-K_α) = 27.8 cm^{-1}.

Intensity measurements were made as above on long, very thin lathes for $\theta \leqslant 22°$ for $h \pm k \pm l$. About 1 300 unique reflections were counted and of these only 762 had $I \geqslant 3\sigma I$. These were used in the structure determination and the refinement. Absorption corrections were not made.

The structure was solved by heavy-atom methods and refined by full-matrix least-squares with C,N,O treated isotropically and bromine treated anisotropically, to a conventional R value of 9.2%. After determination of the absolute configuration further refinement using anomalous dispersion corrections led to convergence at an R value of 8.8%. Computations were done with the XRAY 72 package.

(c) p-*Bromobenzyl isoclavulanate* (**32**): *Crystal data.* $C_{15}H_{14}BrNO_5$, $M = 368.2$, monoclinic, $a = 11.996(2)$, $b = 5.113(1)$, $c = 12.346(2)$ Å, $\beta = 92.92(2)°$, $U = 756.3$ Å^3 (by least-squares refinement of the setting angles of 23 reflections), $D_c = 1.62$ g cm^{-3}, space group $P2_1$, $Z = 2$, μ(Mo-K_α) = 29.1 cm^{-1}.

Intensity measurements were made as above on long, slightly thicker lathes for $\theta \leqslant 22°$ for $h \pm k \pm l$. About 1 250 unique reflections were counted of which 833 had $I > 3\sigma I$. These were used in the structure determination and refinement. Absorption corrections were not made.

The structure was solved by heavy-atom methods and refined by full-matrix least-squares with C,N,O treated isotropically and bromine treated anisotropically, to a conventional R value of 7.9%. The absolute configuration was then determined in the same way as above. In this case the ratio of the weighted R factors was 1.037 (0.0833/0.0803), significant at much below the 0.005 level. Additionally 22 parts of reflections were identified which should have significantly differing counts for the two enantiomers. In every case the observed counts were different and the difference was in the correct sense for the absolute configuration depicted. After determination of the absolute configuration further refinement using anomalous dispersion corrections lead to convergence at a R value of 7.7%. Computations were done with the X-RAY 72 package.

(d) *Compound* (**45**). The structure of this compound was first determined at about the same time as that of the other two structures. The results were of good quality and the final R value was 0.046. Unfortunately the relevant computer files and much of the computer output has been lost making this work unpublishable. So we have recently collected data again in a CAD-4 diffractometer with copper radiation (Ni filter).

Crystal data. $C_{16}H_{15}NO_6$, $M = 317.3$, orthorhombic, $a = 13.479(1)$, $b = 15.227(1)$, $c = 7.031(1)$ Å, $U = 1\,443.0$ Å^3 (from least-squares refinement of the setting angles of 25 reflections), $D_c = 1.46$, $D_m = 1.45$ g cm^{-3}, space group $P2_12_12_1$, $Z = 4$. Crystal size, an approximate cube of side 0.15 mm (Cu-K_α) 19.3 cm^{-1}. 1 739 Reflections were measured ($\theta_{max.} = 76°$) of which 1 583 were observed ($I \geqslant 3\sigma I$) and were used in the refinement. The initial atomic positions were taken from the earlier determination (solved by MULTAN) and refinement was by full-matrix least-squares with C,N,O anisotropic and hydrogens in calculated positions (the alcoholic hydrogen in its found position). The weighting scheme was derived from a four-term Chebyshev series minimising R; R was 0.0395 and R_w 0.047. The ranges of standard deviations for bond lengths and angles was 0.003—0.007 Å (all those above 0.003 were associated with the benzene ring) and 0.2—0.4° (again all those above the minimum were associated with the benzene ring). Refinement was done using the Oxford CRYSTALS package on the UMRCC CDC 7600 computer.

Structure factors and thermal parameters for the crystallographic determinations are deposited as a Supplementary Publication [SUP. No. 23845 (62 pp.)].*

Table 1. Fractional atomic co-ordinates ($\times 10^4$) with standard deviations in parentheses for ester (**14**)

Atom*	*x/a*	*y/b*	*z/c*
O(1)	1 061(1)	9 927	9 438(6)
C(2)	1 591(2)	9 445(5)	7 984(7)
C(3)	1 715(2)	7 522(5)	8 351(6)
N(4)	1 089(2)	6 974(4)	9 379(6)
C(5)	766(2)	8 454(6)	10 567(7)
C(6)	60(2)	7 930(6)	9 176(9)
C(7)	474(2)	6 636(6)	7 734(8)
C(8)	1 905(2)	10 547(6)	6 537(8)
C(9)	1 749(3)	12 453(6)	6 388(10)
C(10)	2 331(2)	7 156(5)	10 387(7)
C(11)	3 542(2)	7 424(5)	11 178(8)
C(12)	3 692(2)	9 031(5)	12 755(7)
C(13)	3 364(2)	10 586(6)	12 091(8)
C(14)	3 529(2)	12 033(5)	13 571(8)
C(15)	4 028(2)	11 938(5)	15 731(8)
C(16)	4 362(2)	10 422(6)	16 439(8)
C(17)	4 191(2)	8 978(6)	14 939(8)
O(7)	377(2)	5 714(5)	5 833(7)
O(9)	2 198(5)	13 339(10)	8 364(21)
O(9a)	1 712(6)	13 017(11)	3 786(17)
O(10)	2 305(2)	6 662(6)	12 595(6)
O(11)	2 918(1)	7 570(4)	9 365(5)
N(15)	4 204(2)	13 461(6)	17 310(8)
O(15)	3 955(3)	14 829(5)	16 575(9)
O(15a)	4 601(2)	13 329(6)	19 329(7)

* Crystallographic numbering system.

Table 2. Bond lengths (Å) with standard deviations in parentheses for ester (**14**)

O(1)–C(2)	1.377(5)	C(10)–O(10)	1.180(5)
O(1)–C(5)	1.427(5)	C(10)–O(11)	1.343(5)
C(2)–C(3)	1.527(5)	O(11)–C(11)	1.450(4)
C(2)–C(8)	1.317(6)	C(11)–C(12)	1.495(5)
C(3)–N(4)	1.440(5)	C(12)–C(13)	1.395(6)
C(3)–C(10)	1.524(5)	C(12)–C(17)	1.392(5)
N(4)–C(5)	1.470(5)	C(13)–C(14)	1.371(6)
N(4)–C(7)	1.412(5)	C(14)–C(15)	1.384(5)
C(5)–C(6)	1.537(5)	C(15)–C(16)	1.379(6)
C(6)–C(7)	1.522(7)	C(15)–N(15)	1.450(6)
C(7)–O(7)	1.195(6)	C(16)–C(17)	1.377(6)
C(8)–C(9)	1.520(7)	N(15)–O(15)	1.214(6)
C(9)–O(9)	1.443(11)	N(15)–O(15a)	1.216(5)
C(9)–O(9a)	1.375(10)		

* For details of the Supplementary Publications Scheme, see Instructions for Authors (1984), *J. Chem. Soc., Perkin Trans. 1*, 1984, Issue 1.

Table 3. Bond angles (°) with standard deviations in parentheses for ester (**14**)

C(2)-O(1)-C(3)	110.3(3)	C(8)-C(9)-O(9a)	110.3(5)
O(1)-C(2)-C(3)	108.8(3)	C(3)-C(10)-O(10)	125.9(3)
O(1)-C(2)-C(8)	122.2(4)	C(3)-C(10)-O(11)	110.1(3)
C(3)-C(2)-C(8)	129.0(4)	O(10)-C(10)-O(11)	123.9(3)
C(2)-C(3)-N(4)	101.8(3)	C(10)-O(11)-C(11)	115.9(3)
C(2)-C(3)-C(10)	111.7(3)	O(11)-C(11)-C(12)	111.9(3)
C(10)-C(3)-N(4)	110.2(3)	C(11)-O(12)-C(13)	122.8(3)
C(3)-N(4)-C(5)	109.1(3)	C(11)-C(12)-C(17)	118.4(3)
C(3)-N(4)-C(7)	123.3(3)	C(13)-C(12)-C(17)	118.8(4)
C(5)-N(4)-C(7)	90.6(3)	C(12)-C(13)-C(14)	120.6(4)
N(4)-C(5)-O(1)	105.3(3)	C(13)-C(14)-C(15)	119.3(4)
N(4)-C(5)-C(6)	90.5(3)	C(14)-C(15)-C(16)	121.6(4)
O(1)-C(5)-C(6)	114.4(3)	C(14)-C(15)-N(15)	119.6(4)
C(5)-C(6)-C(7)	84.1(3)	C(16)-C(15)-N(15)	118.7(3)
C(6)-C(7)-N(4)	93.4(3)	C(15)-C(16)-C(17)	118.5(4)
C(6)-C(7)-O(7)	137.0(4)	C(16)-C(17)-C(12)	121.3(4)
N(4)-C(7)-O(7)	129.5(4)	C(15)-N(15)-O(15)	119.1(4)
C(2)-C(8)-C(9)	123.8(4)	C(15)-N(15)-O(15a)	118.9(4)
C(8)-C(9)-O(9)	108.7(5)	O(15)-N(15)-O(15a)	122.0(5)

Table 4. Fractional atomic co-ordinates ($\times 10^3$) with standard deviations in parentheses for ester (**15**)

Atom*	*x/a*	*y/b*	*z/c*
O(1)	216(1)	636(3)	770(1)
C(2)	241(1)	798(5)	681(1)
C(3)	356(1)	778(6)	667(1)
N(4)	390(1)	601(4)	765(1)
C(5)	302(1)	464(5)	815(1)
C(6)	345(2)	598(5)	926(2)
C(7)	418(1)	763(6)	863(2)
C(8)	175(1)	950(5)	622(1)
C(9)	67(2)	996(6)	650(2)
C(10)	372(1)	560(5)	571(1)
C(11)	346(1)	534(6)	384(2)
C(12)	289(1)	731(6)	299(1)
C(13)	346(2)	882(5)	234(2)
C(14)	294(2)	1 050(5)	154(2)
C(15)	191(2)	1 042(5)	139(2)
C(16)	132(2)	883(5)	207(2)
C(17)	183(1)	714(6)	289(2)
O(7)	480(1)	946(4)	872(1)
O(9)	−3(1)	961(4)	552(1)
O(10)	413(1)	340(4)	576(1)
O(11)	338(1)	695(3)	483(1)
Br(1)	120.1(2)	1 250	30.8(2)

* Crystallographic numbering system.

Table 5. Bond lengths (Å) with standard deviations in parentheses for ester (**15**)

O(1)-C(2)	1.43(4)	C(9)-O(9)	1.48(4)
O(1)-C(5)	1.46(3)	C(10)-O(10)	1.18(3)
C(2)-C(3)	1.53(2)	C(10)-O(11)	1.33(4)
C(2)-C(8)	1.31(3)	O(11)-C(11)	1.49(5)
C(3)-N(4)	1.53(5)	C(11)-C(12)	1.56(4)
C(3)-C(10)	1.63(5)	C(12)-C(13)	1.39(4)
N(4)-C(5)	1.52(3)	C(12)-C(17)	1.39(3)
N(4)-C(7)	1.48(5)	C(13)-C(14)	1.42(4)
C(5)-C(6)	1.60(6)	C(14)-C(15)	1.34(3)
C(6)-C(7)	1.53(4)	C(15)-C(16)	1.43(4)
C(7)-O(7)	1.19(3)	C(15)-Br(1)	1.87(4)
C(8)-C(9)	1.52(3)	C(16)-C(17)	1.43(4)

Table 6. Bond angles (°) with standard deviations in parentheses for ester (**15**)

C(2)-O(1)-C(5)	113(2)	C(2)-C(8)-C(9)	122(2)
O(1)-C(2)-C(3)	111(1)	C(8)-C(9)-O(9)	109(2)
O(1)-C(2)-C(8)	124(2)	C(3)-C(10)-O(10)	129(2)
C(3)-C(2)-C(8)	125(2)	C(3)-C(10)-O(11)	105(3)
C(2)-C(3)-N(4)	99(1)	O(10)-C(10)-O(11)	126(2)
C(2)-C(3)-C(10)	109(1)	C(10)-O(11)-C(11)	114(3)
C(10)-C(3)-N(4)	102(3)	O(11)-C(11)-C(12)	102(3)
C(3)-N(4)-C(5)	113(2)	C(11)-C(12)-C(13)	118(2)
C(3)-N(4)-C(7)	115(3)	C(11)-C(12)-C(17)	117(2)
C(5)-N(4)-C(7)	91(2)	C(13)-C(12)-C(17)	125(2)
N(4)-C(5)-O(1)	101(2)	C(12)-C(13)-C(14)	118(2)
N(4)-C(5)-C(6)	88(2)	C(13)-C(14)-C(15)	120(2)
O(1)-C(5)-C(6)	109(2)	C(14)-C(15)-C(16)	122(2)
C(5)-C(6)-C(7)	86(2)	C(14)-C(15)-Br(1)	117(2)
C(6)-C(7)-N(4)	92(3)	C(16)-C(15)-Br(1)	117(2)
C(6)-C(7)-O(7)	142(2)	C(15)-C(16)-C(17)	119(2)
N(4)-C(7)-O(7)	126(2)	C(16)-C(17)-C(12)	116(2)

Table 7. Fractional atomic co-ordinates ($\times 10^4$) with standard deviations in parentheses for photo-product (**45**).

Atom*	*x/a*	*y/b*	*z/c*	U_{iso}
O(1)	7 185(1)	7 199(1)	−606(2)	
C(2)	6 852(2)	6 945(1)	1 228(3)	
C(3)	7 767(2)	6 576(1)	2 287(3)	
N(4)	8 439(1)	6 393(1)	745(3)	
C(5)	8 193(2)	6 946(2)	−905(3)	
C(6)	8 370(2)	6 154(2)	−2 207(3)	
C(7)	8 440(2)	5 638(2)	−365(3)	
C(8)	5 866(2)	6 427(2)	1 232(3)	
C(9)	5 482(2)	6 096(2)	−645(4)	
C(10)	8 216(2)	7 251(2)	3 643(3)	
C(11)	6 586(2)	7 519(2)	4 448(4)	
C(12)	6 250(2)	7 653(2)	2 351(3)	
C(13)	6 234(2)	8 585(2)	1 670(4)	
C(14)	6 558(2)	9 279(2)	2 809(6)	
C(15)	6 515(3)	10 143(2)	2 077(8)	
C(16)	6 149(3)	10 301(2)	296(9)	
C(17)	5 834(2)	9 609(2)	−847(6)	
C(18)	5 878(2)	8 759(2)	−164(5)	
O(7)	8 466(1)	4 875(1)	105(3)	
O(9)	6 034(2)	5 320(1)	−1 035(3)	
O(10)	9 075(1)	7 413(1)	3 747(3)	
O(11)	7 561(1)	7 681(1)	4 762(2)	
O(12)	5 330(1)	7 187(1)	1 936(3)	
H(1)	6 036	5 311	−2 322	0.0500
H(2)	7 624	6 037	3 075	0.0500
H(3)	8 495	7 528	−1 239	0.0500
H(4)	7 795	5 992	−3 066	0.0500
H(5)	8 988	6 175	−2 998	0.0500
H(6)	5 817	5 859	1 959	0.0500
H(7)	5 580	6 544	−1 665	0.0500
H(8)	4 749	5 955	−557	0.0500
H(9)	6 107	7 936	5 246	0.0500
H(10)	6 344	6 901	4 834	0.0500
H(11)	6 824	9 161	4 126	0.0500
H(12)	6 773	10 648	2 853	0.0500
H(13)	6 116	10 916	−177	0.0500
H(14)	5 556	9 735	−2 141	0.0500
H(15)	5 642	8 260	995	0.0500

* Crystallographic numbering system.

Table 8. Bond lengths (Å) with standard deviations in parentheses for photo-product (**45**)

O(1)–C(2)	1.420(3)	C(9)–O(9)	1.422(3)
O(1)–C(5)	1.428(3)	C(10)–O(10)	1.187(3)
C(2)–C(3)	1.547(3)	C(10)–O(11)	1.351(3)
C(2)–C(8)	1.545(3)	C(11)–C(12)	1.528(3)
C(2)–C(12)	1.564(3)	C(11)–O(11)	1.461(3)
C(3)–N(4)	1.441(3)	C(12)–C(13)	1.497(3)
C(3)–C(10)	1.527(3)	C(12)–O(12)	1.459(3)
N(4)–C(5)	1.472(3)	C(13)–C(14)	1.396(4)
N(4)–C(7)	1.389(3)	C(13)–C(18)	1.452(4)
C(5)–C(6)	1.532(3)	C(14)–C(15)	1.413(5)
C(6)–C(7)	1.518(3)	C(15)–C(16)	1.367(7)
C(7)–O(7)	1.208(3)	C(16)–C(17)	1.392(6)
C(8)–C(9)	1.504(3)	C(17)–C(18)	1.382(4)
C(8)–O(12)	1.452(3)		

Table 9. Bond angles (°) with standard deviations in parentheses for photo-product (**45**)

C(2)–O(1)–C(5)	111.2(2)	C(9)–C(8)–O(12)	113.3(2)
O(1)–O(2)–C(3)	106.5(2)	C(8)–C(9)–O(9)	105.5(2)
O(1)–C(2)–C(3)	114.4(2)	C(3)–C(10)–O(10)	124.4(2)
O(1)–C(2)–C(12)	115.7(2)	C(3)–C(10)–O(11)	115.5(2)
C(3)–C(2)–C(8)	120.0(2)	O(10)–C(10)–O(11)	120.0(2)
C(3)–C(2)–C(12)	114.9(2)	C(12)–C(11)–O(11)	110.0(2)
C(8)–C(2)–C(12)	84.5(2)	C(2)–C(12)–C(11)	106.1(2)
C(2)–C(3)–N(4)	102.1(2)	C(2)–C(12)–C(13)	120.0(2)
C(2)–C(3)–C(10)	111.8(2)	C(2)–C(12)–O(12)	90.2(2)
N(4)–C(3)–C(10)	110.5(2)	C(11)–C(12)–C(13)	116.0(2)
C(3)–N(4)–C(5)	109.9(2)	C(11)–C(12)–O(12)	108.6(2)
C(3)–N(4)–C(7)	125.7(2)	C(13)–C(12)–O(12)	112.7(2)
C(5)–N(4)–C(7)	91.8(2)	C(12)–C(13)–C(14)	122.0(3)
O(1)–C(5)–N(4)	104.7(2)	C(12)–C(13)–C(18)	118.6(3)
O(1)–C(5)–C(6)	116.7(2)	C(14)–C(13)–C(18)	119.5(3)
N(4)–C(5)–C(6)	89.1(2)	C(13)–C(14)–C(15)	118.8(4)
C(5)–C(6)–C(7)	84.7(2)	C(14)–C(15)–C(16)	120.9(4)
N(4)–C(7)–C(6)	92.9(2)	C(15)–C(16)–C(17)	120.3(3)
N(4)–C(7)–O(7)	129.9(2)	C(16)–C(17)–C(18)	119.7(4)
C(6)–C(7)–O(7)	137.1(2)	C(13)–C(18)–C(17)	120.7(3)
C(2)–C(8)–C(9)	117.7(2)	C(10)–O(11)–C(11)	117.8(2)
C(2)–C(8)–O(12)	91.3(2)	C(8)–O(12)–C(12)	91.9(2)

Table 10. Fractional atomic co-ordinates ($\times 10^3$) with standard deviations in parentheses for ester (**28**)

Atom*	*x/a*	*y/b*	*z/c*
O(1)	199(1)	393(3)	701(1)
C(2)	209(1)	209(4)	785(1)
C(3)	334(1)	193(4)	828(1)
N(4)	386(1)	315(3)	733(1)
C(5)	305(1)	494(4)	669(1)
C(6)	347(1)	364(4)	564(1)
C(7)	411(1)	175(4)	638(1)
C(8)	120(1)	75(4)	817(1)
C(9)	126(1)	−124(4)	901(1)
C(10)	357(1)	385(4)	926(1)
C(11)	334(2)	415(4)	1 116(2)
C(12)	280(1)	234(5)	1 199(1)
C(13)	167(2)	301(6)	1 217(1)
C(14)	114(2)	124(5)	1 297(2)
C(15)	175(1)	−46(4)	1 353(1)
C(16)	287(2)	−100(5)	1 334(2)
C(17)	339(2)	54(5)	1 257(2)
O(7)	464(1)	−12(3)	633(1)
O(9)	40(1)	−53(3)	980(1)
O(10)	398(1)	586(3)	925(1)
O(11)	321(1)	252(4)	1 013(1)
Br(1)	105.0(2)	−1 250	1 457.6(2)

*Crystallographic numbering system.

Table 11. Bond lengths (Å) with standard deviations in parentheses for ester (**28**)

O(1)–C(2)	1.40(2)	C(9)–O(9)	1.50(2)
O(1)–C(5)	1.44(2)	C(10)–O(10)	1.14(3)
C(2)–C(3)	1.56(2)	C(10)–O(11)	1.37(2)
C(2)–C(8)	1.35(3)	O(11)–C(11)	1.52(2)
C(3)–N(4)	1.49(2)	C(11)–C(12)	1.55(3)
C(3)–C(10)	1.57(3)	C(12)–C(13)	1.35(3)
N(4)–C(6)	1.53(2)	C(12)–C(17)	1.44(3)
N(4)–C(7)	1.42(2)	C(13)–C(14)	1.41(3)
C(5)–C(6)	1.57(3)	C(14)–C(15)	1.40(3)
C(6)–C(7)	1.52(3)	C(15)–C(16)	1.31(3)
C(7)–O(7)	1.15(3)	C(15)–Br(1)	1.89(2)
C(8)–C(9)	1.45(3)	C(16)–C(17)	1.50(3)

Table 12. Bond angles (°) with standard deviations in parentheses for ester (**28**)

C(2)–O(1)–C(5)	113(1)	C(2)–C(8)–C(9)	124(2)
O(1)–C(2)–C(3)	109(1)	C(8)–C(9)–O(9)	106(2)
O(1)–C(2)–C(8)	121(1)	C(3)–C(10)–O(10)	128(2)
C(3)–C(2)–C(8)	129(2)	C(3)–C(10)–O(11)	104(2)
C(2)–C(3)–N(4)	98(1)	O(10)–C(10)–O(11)	128(2)
C(2)–C(3)–C(10)	111(1)	O(10)–O(11)–C(11)	111(2)
C(10)–C(3)–N(4)	106(1)	O(11)–C(11)–C(12)	101(2)
C(3)–N(4)–C(5)	112(1)	C(11)–C(12)–C(13)	123(2)
C(3)–N(4)–C(7)	124(2)	C(11)–C(12)–C(17)	113(2)
C(5)–N(4)–C(7)	92(1)	C(13)–C(12)–C(17)	124(2)
N(4)–C(5)–O(1)	101(1)	C(12)–C(13)–C(14)	120(2)
N(4)–C(5)–C(6)	87(1)	C(13)–C(14)–C(15)	118(2)
O(1)–C(5)–C(6)	114(2)	C(14)–C(15)–C(16)	124(2)
C(5)–C(6)–C(7)	87(1)	C(14)–C(15)–Br(1)	118(1)
C(6)–C(7)–N(4)	93(1)	C(16)–C(15)–Br(1)	118(2)
C(6)–C(7)–O(7)	140(2)	C(15)–C(16)–C(17)	121(2)
C(4)–C(7)–O(7)	127(2)	C(16)–C(17)–C(12)	113(2)

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