Tetrahedron Vol. 39, No. 15, pp. 2551 to 2562, 1983
Printed in Great Britain.

0040-4020/83 $3.00 + .00
Pergamon Press Ltd.

STUDIES RELATED TO THE STRUCTURES OF THE OLIVANIC ACIDS MM 13902, MM 4550 AND MM 17880

THREE β-LACTAM ANTIBIOTICS FROM *STREPTOMYCES OLIVACEUS*

ALLAN G. BROWN, DAVID F. CORBETT,* A. JOHN EGLINGTON and T. TREFOR HOWARTH
Beecham Pharmaceuticals, Research Division, Brockham Park, Betchworth, Surrey RH3 7AJ, England

(*Received in UK* 20 *August* 1982)

Abstract—MM 13902, a β-lactam antibiotic isolated from *Streptomyces olivaceus*, has been identified as (5*R*,6*R*) - 3 - [(*E*) - 2 - acetamidoethenylthio] - 6 - [(*S*) - 1 - hydroxysulphonyloxyethyl] - 7 - oxo - 1 - azabicyclo[3.2.0]hept - 2 - ene - 2 - carboxylic acid (**2**) by chemical and spectroscopic studies. The related olivanic acids MM 4550 and MM 17880 have been shown to be the corresponding 3 - [(*E*) - 2 - acetamidoethenylsulphinyl]- and 3 - (2 - acetamidoethylthio)- derivatives (**1** and **5**), respectively. A number of carboxylate and sulphate esters of **1**, **2** and **5** have been prepared. A key reaction in the characterisation of **2** was the oxidative degradation of its C-2 monomethyl ester (**12**) in dimethylsulphoxide to methyl 3 - [(*E*) - 2 - acetamidoethenylthio] - 5 - [(1*R*,2*S*) - 1 - carboxy - 2 - hydroxysulphonyloxyprop - 1 - yl]pyrrole - 2 - carboxylate (**22**). The configuration of the C-6 substituent of **2** was determined by a stereospecific elimination reaction of its ethyl sulphate, *p*-nitrobenzyl carboxylate (**19**) to yield the 6 - (*E*) - ethylidene derivative (**32**). MM 13902 was oxidised to MM 4550 with *m*-chloroperbenzoic acid.

The remarkable ability of micro-organisms to produce biologically active compounds with novel and unexpected structures has been a constant fascination to chemists for many years. Nowhere has this been more evident than in the recent discovery of a new class of β-lactam antibiotics produced by various *Streptomyces* species and possessing the characteristic 7 - oxo - 1 - azabicyclo[3.2.0]hept - 2 - ene ring system. As a result of two independent investigations, the earliest metabolites of this type to be isolated and characterised were the olivanic acids[1,2] from *Streptomyces olivaceus* and thienamycin[3] from *Streptomyces cattleya*. More recently, a proliferation of related structures has emerged, and these include the carpetimycins,[4] asparenomycins,[5] and PS-5 family of antibiotics.[6] The diversity of structures within this new class of fused-bicyclic β-lactam antibiotics has prompted considerable interest in the area, providing a challenge for natural product and synthetic chemist alike.

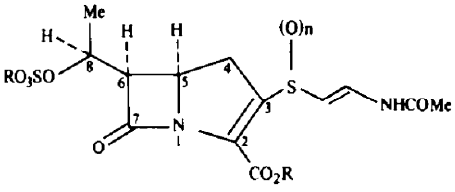
The olivanic acids consist of a family of naturally occurring compounds which possess potent antibacterial activity and β-lactamase inhibitory properties.[7,8] The first three metabolities to be isolated were the sulphate derivatives, designated MM 4550[1] (**1**), MM 13902[1] (**2**) and MM 17880[2] (**5**). Subsequently, four new related products were discovered, these being the non-sulphated analogues MM 22380 (**8**), MM 22381 (**10**), MM 22382 (**9**) and MM 22383 (**11**).[8,9] More recently, the isolation of a side-chain homologue of MM 13902, the propionamido-derivative (**7**) has also been reported.[10] We now describe some of the chemistry involved in the structure elucidation of the first three olivanic acids MM 4550, MM 13902 and MM 17880. These early studies were hindered by the poor chemical stability of the compounds and their derivatives, and by the very limited quantities available at the time.

MM 13902 (**2**) was isolated as its freeze-dried sodium salt (**3**) which possessed spectroscopic properties (UV, IR, ^{1}H NMR, ^{13}C NMR) in accord with the proposed structure.[1] However, the data were not sufficiently diagnostic to determine the stereochemistry at C-8 of the molecule, whereas the relative configuration between the C-5 and C-6 protons was determined to be *cis* by virtue of the ^{1}H NMR coupling constant ($J_{5,6}$ = 5.5 Hz). Reaction of **3** with various alkyl halides in N,N-dimethylformamide (DMF) afforded a series of monoesters (**12–15**), the spectral data of which supported the structural assignments. These esters also provided additional evidence for the molecular composition of MM 13902 by means of analytical data and field desorption mass spectrometry studies.[1] The esters did not exhibit molecular ions, but significant fragmentations were observed, which confirmed part of the structure including the sulphate moiety. Inconveniently, these monoesters had good solubility only in polar solvents such as DMF, water and dimethylsulphoxide (DMSO) but the use of chloroform–ethanol mixtures was found to be expedient, despite poor solubility in either solvent alone. Such solvent mixtures were of particular value for chromatography.

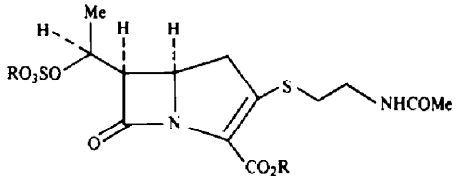
More vigorous alkylating conditions were required to prepare sulphate esters of MM 13902; thus acidification of the disodium salt (**3**) with *p*-toluenesulphonic acid followed by immediate reaction with diazomethane led to the formation of the dimethyl ester (**16**). This derivative proved to be very labile but could be isolated in low yield by rapid silica-gel chromatography. The significance of the diester (**16**) was that it became the first derivative of MM 13902 to show a molecular ion (*m/e* 420) in the field desorption mass spectrum. A more stable series of diesters were the ethyl sulphates, conveniently prepared by alkylation of the sodium sulphates with Meerwein's reagent ($Et_3O^+ BF_4^-$). Thus, reaction of the mono-ester (**13**) with triethyloxonium tetrafluoroborate gave the diester (**17**) as a stable crystalline solid. The preparation of diesters was

facilitated by first converting the monoester monosodium salts of MM 13902 into monoquaternary ammonium salts thus rendering them soluble in common organic solvents. Thus, reaction of the monoesters (**12** and **14**) with benzyldimethyl-n-hexadecylammonium chloride afforded the quaternary ammonium salts (**20** and **21**) which were subsequently alkylated with Meerwein's reagent to furnish the diesters (**18** and **19**), respectively.

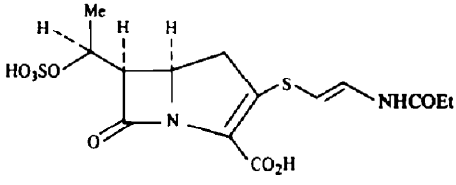
The main chemical evidence in support of the structure assigned to MM 13902 came from an oxidative β-lactam degradation reaction of the monomethyl ester (**12**) to afford a pyrrole derivative.[2] When **12** was heated for 2 hr, or allowed to stand at room temp. for 3 days, in DMSO an essentially quantitative transformation occurred to give the pyrrole (**22**). This conversion could be conveniently monitored by ^1H or ^{13}C NMR spectroscopy by performing the reaction in an NMR tube using deuterated solvent. Of particular significance was the observed disappearance of signals due to the 4-CH_2 and 5-CH of the β-lactam (**12**) with the concomitant appearance of a pyrrole β-CH resonance. The pyrrole (**22**) was further characterised by successive



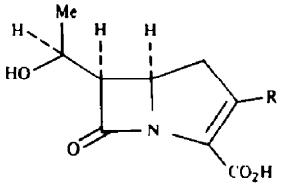
(1) n = 1, R = H
(2) n = 0, R = H
(3) n = 0, R = Na
(4) n = 1, R = Na



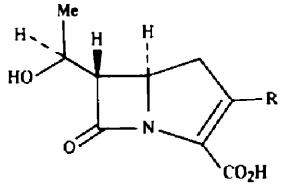
(5) R = H
(6) R = Na



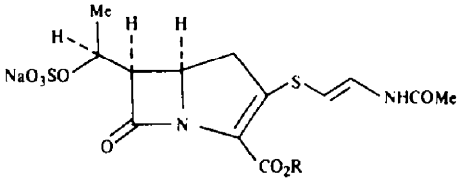
(7)



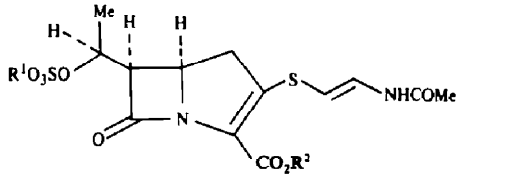
(8) R = $SCH_2CH_2NHCOMe$
(9) R = S⟨ ⟩NHCOMe



(10) R = $SCH_2CH_2NHCOMe$
(11) R = S⟨ ⟩NHCOMe



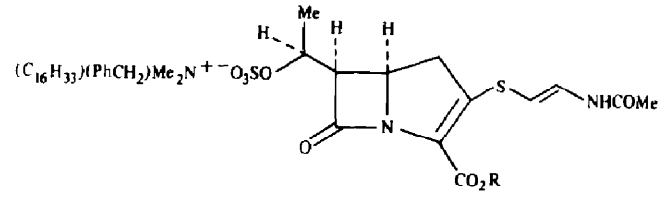
(12) R = Me
(13) R = CH_2—⟨ ⟩—Br
(14) R = CH_2—⟨ ⟩—NO_2 (PNB)
(15) R = CH_2 C(=O)—⟨ ⟩—Br



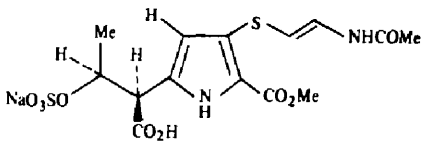
(16) $R^1 = R^2$ = Me
(17) R^1 = Et, R^2 = CH_2—⟨ ⟩—Br
(18) R^1 = Et, R^2 = Me
(19) R^1 = Et, R^2 = PNB

esterification of the acid with diazomethane and elimination of the elements of sodium hydrogen sulphate with 1,8-diazobicyclo[5.4.0]undec-7-ene (DBU) to produce the isolable pyrrole (**23**). Although the latter was non-crystalline and consisted of an inseparable mixture (*ca* 5 : 1) of (*E*)- and (*Z*)-ethylidene isomers, it was fully characterised by spectroscopic data with the mass spectral fragmentation pattern being particularly informative.[2] Desulphurisation of **23** was effected by the use of Raney nickel in ethanol, although reduction of the olefinic double bond and transesterification occurred simultaneously to yield the new pyrrole (**24**). Attempts to effect a similar removal of the C-3 sulphur substituent of the intact bicyclic system resulted in decomposition with loss of the β-lactam ring.

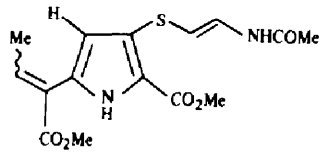
As the ester (**12**) did not undergo transformation to the pyrrole (**22**) in either DMF or water, it would suggest that the DMSO plays an integral part in the reaction. A suggested mechanism is shown in Scheme 1. Initial opening of the β-lactam ring by DMSO is followed by nucleophilic reaction of the resulting nitrogen anion on the cationic S atom with the liberation of a carboxylate function. The intermediate (**26**) is then able to undergo a facile elimination of dimethyl sulphide to form the pyrrole (**22**). The reaction is remarkably sensitive to changes of substituent in the 5-membered ring. For instance, the corresponding sulphoxide (**27**) and the *p*-nitrobenzyl ester (**14**) did not produce pyrroles on heating in DMSO, whilst the *p*-bromobenzyl ester (**13**) readily underwent the transformation. A possible explanation of



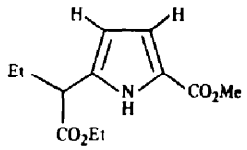
(20) R = Me
(21) R = PNB



(22)

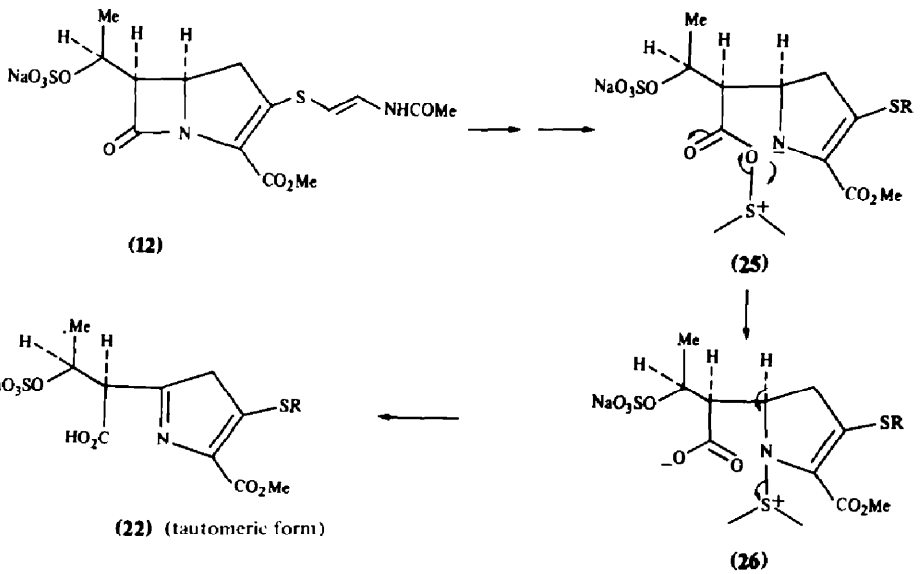


(23)



(24)

Scheme 1.



(12)

(25)

(22) (tautomeric form)

(26)

these observations is that electron-withdrawing substituents such as the sulphoxide in **27** or the ester in **14** render the nitrogen anion in **25** less nucleophilic and prevent the formation of the intermediate (**26**). In the presence of water (in DMSO) **25** would then lead to hydrolysis products in which the β-lactam ring has opened but oxidation to a pyrrole has not occurred.

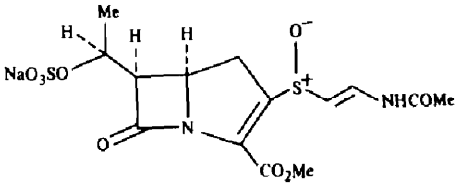
Hydrolysis and alcoholysis reactions of MM 13902 and its derivatives have been investigated with limited success. Both acidic and basic hydrolysis of the disodium salt of MM 13902 (**3**) proved to be complex and the presence of the sulphate moiety hindered efforts to identify the products formed. However, base-induced methanolysis reactions of monoesters of MM 13902 were somewhat more fruitful. The reaction of the monomethyl ester (**12**) with diethylamine in methanol led to the isolation of sodium sulphate and two organic products. The least polar of these was characterised as the hydroxypyrrole (**28**), which was isolated as a mixture (*ca* 7 : 1) of (*E*) and (*Z*)-isomers. Spectroscopic data were of paramount importance in establishing this structure; the chemical shift of the pyrrole proton (δ 5.92) in the ^{1}H NMR spectrum is consistent only with a pyrrole β-CH which is not adjacent to an α-ester function;[11] the IR ester absorption at 1665 cm^{-1} is indicative of a chelated CO group; and a molecular ion at *m/e* 239.0795 in the mass spectrum confirmed the molecular formula as $C_{11}H_{13}NO_5$. The more polar product was water-soluble and although not fully characterised was considered to be the Δ^1-pyrroline (**29**). Its IR spectrum (ν_{max} 1730, 1675, 1620 and 1270–1210 cm^{-1}) indicated that the original C-3 side-chain and the sulphate were still present, and the β-lactam ring had cleaved. The ^{1}H NMR spectrum was more in accord with the Δ^1-pyrroline (**29**) than the Δ^2-analogue (**30**) which would be formed from simple methanolysis of the β-lactam, and it is in fact well known that the latter type of pyrrolines are unstable species which isomerise to the Δ^1-derivatives.[12] The pyrroline (**29**) was very slowly converted to the hydroxypyrrole (**28**) with diethylamine but failed to oxidise to a pyrrole in DMSO. The latter observation precludes hydrolysis of the β-lactam followed by oxidation in the DMSO reaction as an alternative process to the proposed mechanism in Scheme 1. A similar methanolysis reaction of **12** using one equivalent of sodium methoxide as the base afforded the same two products **28** (6%) and **29** (65%) but this time the latter existed as two diastereoisomers, epimeric at the pyrroline-CH adjacent to S. The hydroxypyrrole (**28**) must arise from a hydrolytic cleavage of the S side-chain and an oxidation step, but more detailed mechanistic conjecture would be inappropriate in the absence of further data. The isolation of **28** provided the first chemical evidence that the structure of MM 13902 proposed on the basis of spectroscopic data was plausible. A related hydroxypyrrole has been obtained from a degradation reaction of thienamycin.[3]

The occurrence of base-induced elimination of sodium hydrogen sulphate in the afore-mentioned β-lactam degradation reactions prompted us to investigate the possibility of a similar reaction in the intact bicyclic system. It was considered that a non-nucleophilic base in an aprotic solvent would be necessary to minimise the chance of β-lactam ring-opening. The monomethyl ester of MM 13902 (**12**) was thus treated with anhydrous potassium carbonate in DMF (20 hr, r.t. or 4 hr, 70°) and indeed the expected elimination occurred to yield the ethylidene derivative as a mixture (*ca* 3 : 1) of (*E*)-isomer (**31**) and (*Z*)-isomer (**33**) (60%). The isomers were distinguishable by the ^{1}H NMR chemical shifts of the olefinic protons and methyl substituents, which were influenced by the deshielding effect of the β-lactam CO group. Consequently, the olefinic proton in the (*E*)-isomer (**31**), appeared at *ca* 0.5 ppm downfield from that of the (*Z*)-isomer (**33**), whilst the Me signal of the latter was a similar value downfield from that of the former. The mixture of isomers appeared as a single component by TLC, but it was possible to isolate a small quantity of each separate isomer by careful fractionation on a silica-gel column.

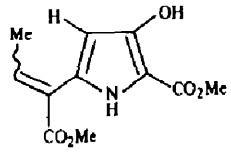
A minor product (4%) obtained from the elimination reaction was identified as the Δ^3-derivative (**35**) [ratio of (*E*) and (*Z*)-isomers = *ca* 3 : 1]. The mass spectrum of **35** showed it to have the same molecular composition as the ethylidene (**31**) [M^+, 308.0832], and other spectroscopic data were consistent with migration of the endocyclic double bond from the Δ^2- to the Δ^3-position of the 5-membered ring. The configuration at C-2 was assumed to be (*S*) in view of the thermodynamic preference of this configuration in the related penicillin and oxapenam systems.[13] The movement of the double bond out of conjugation with the C-2 ester is presumably compensated by a relief of ring-strain in the system as suggested by a lowering of the IR β-lactam CO stretching frequency by *ca* 10 cm^{-1} in **35** compared to **31**. The Δ^2- and Δ^3-ethylidene derivatives were shown to be in equilibrium under the reaction conditions by treating either one with K_2CO_3 in DMF and observing the formation of the same equilibrium mixture in each case. Equilibria between Δ^2- and Δ^3-isomers in olivanic acid[14] and thienamycin analogues[15] have been observed elsewhere. An alternative and generally more convenient procedure for obtaining the C-6 ethylidene derivatives involved prior conversion of monosodium salts of MM 13902 into mono-quaternaryammonium salts, which then underwent elimination with DBU. This process was particularly amenable to the presence of a *p*-nitrobenzyl ester moiety as this underwent partial cleavage with K_2CO_3-DMF resulting in much reduced yields. Thus, the monoester of MM 13902 (**14**), after conversion to the salt (**21**), upon treatment with DBU (CH_2Cl_2, 5°, 4 hr) gave the ethylidene derivatives (**32** and **34**) (*ca* 1 : 1.3) in 57% yield. Again the Δ^3-isomer (**36**) was formed in low yield. (6%)

The formation of mixtures of (*E*)- and (*Z*)-ethylidene isomers in these reactions implied that the elimination of hydrogen sulphate was non-stereospecific and no conclusions about the C-8 stereochemistry of MM 13902 could therefore be reached. The ionic sulphate moiety is presumably a poor leaving group, but the alkyl sulphate present in the diesters (**16**–**19**) is more akin to functions with good leaving ability such as mesylate, and indeed, under suitable conditions, a rapid stereospecific E2 elimination reaction was observed. Thus, the diester (**18**) when treated with potassium acetate in ethanol gave rise to the (*E*)-ethylidene isomer (**31**), exclusivly. Similarly, the *p*-nitrobenzyl, ethyl diester (**19**) under

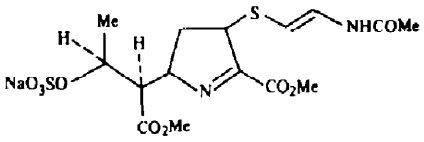
the same conditions or with K_2CO_3-DMF (0.5 hr, r.t.) gave only the (*E*)-isomer (**32**). As the leaving group and β-H atom normally assume an *anti*-coplanar orientation in such stereospecific E2 elimination reactions, and the stereochemistry of MM 13902 was known to be (5*R*,6*R*), it was possible to deduce that the configuration at C-8 was (*S*). At this stage we had assumed that the absolute configuration at C-5 in MM 13902 was (*R*) by analogy with all other naturally occurring β-lactam antibiotics known at the time. Since then the absolute stereochemistry of the olivanic acid MM 22383,[16] thienamycin,[3] the carpetimycins[4] and asparenomycin[5] have all been shown to be (5*R*). Since MM 22382 which is produced in the same fermentation as MM 22383 has been biosynthetically linked to MM 13902,[8] there is little doubt that MM 13902 does indeed possess this configuration. The relative stereochemistry of the three asymmetric centres in MM 13902 was subsequently confirmed by an X-ray analysis of the methyl, ethyl diester[17] (**18**). Previously, apparently good crystals of the *p*-bromobenzyl (**13**) and *p*-bromophenacyl (**15**) esters, had failed to yield structures from this technique, but rather modest crystals of the diester (**18**), despite undergoing some decomposition in the X-ray beam, successfully furnished a



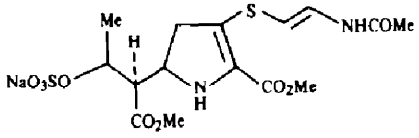
(**27**)



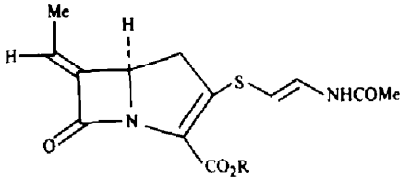
(**28**)



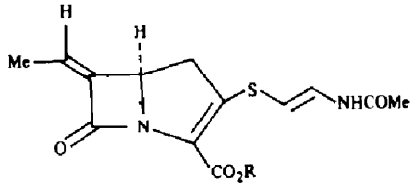
(**29**)



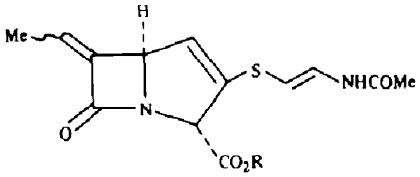
(**30**)



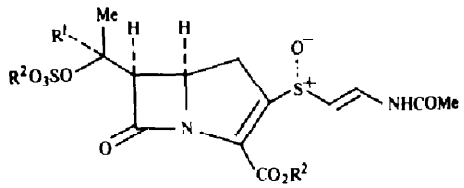
(**31**) R = Me
(**32**) R = PNB



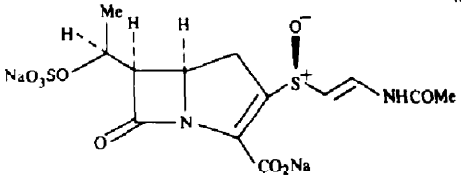
(**33**) R = Me
(**34**) R = PNB



(**35**) R = Me
(**36**) R = PNB



(**37**) R^1 = H, R^2 = Na
(**38**) R^1 = H, R^2 = H
(**39**) R^1 = Me, R^2 = H



(**40**)

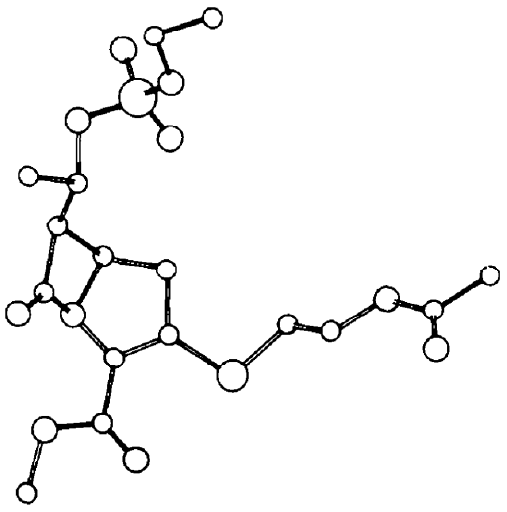


Fig. 1. X-ray structure of the MM 13902 diester (18).

structure. Figure 1 shows the X-ray structure of the molecule, confirming the (5*R*,6*R*,8*S*) relative stereochemistry.

Characterisation of the second metabolite, MM 4550 (**1**) proved more difficult because of its greater chemical instability compared to MM 13902. Its derivatives and degradation products were not generally obtained in a pure form owing to their poor stability and hygroscopic nature. Spectroscopic data for the disodium salt of MM 4550[1] (**4**) were thus of greatest value in the structure determination, in view of their close resemblance to those possessed by MM 13902. The most significant differences in these properties were the lower UV absorption maximum of MM 4550, being 287 nm compared to 308 nm in MM 13902, and chemical shift differences of the side-chain olefinic protons and C atoms in the ^1H and ^{13}C NMR spectra, respectively. As spectroscopic evidence precluded the possibility of MM 4550 being a structural isomer of MM 13902,[1] the likelihood of it possessing the sulphoxide structure (**1**) became evident. Confirmation of this relationship was achieved by oxidation of the disodium salt of MM 13902 with *m*-chloroperbenzoic acid in water to give a mixture (*ca* 1.25 : 1) of two diastereoisomeric sulphoxides (**37** and **40**). The mixture was identical to MM 4550 disodium salt (**4**) by TLC and IR and UV spectroscopy and the major isomer was identical to (**4**) by HPLC and ^1H NMR spectroscopy. The CD spectrum of MM 4550 disodium salt (**4**) showed Cotton effects at 210, 234, 261 and 289 nm whose sign and magnitude are in close agreement with those later reported for the homologue Carpetimycin B[4] (**39**). Since the latter has been established to possess the (*R*)-sulphoxide configuration by X-ray analysis it would seem likely that MM 4550 is also the (*R*)-sulphoxide and possesses structure (**38**). Asparenomycin A has also been reported to have the (*R*)-configuration at the side-chain sulphoxide.[5]

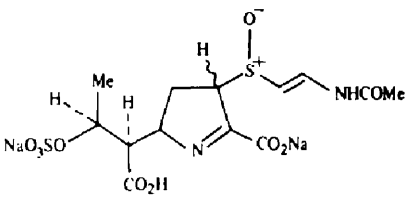
The hydrolysis of the disodium salt of MM 4550 (**4**) in neutral aqueous solution is worthy of note as it resulted in a much cleaner product than the analogous degradation of MM 13902. Opening of the β-lactam ring was evident by the loss of the characteristic olivanic acid UV absorption of MM 4550 (287 nm) accompanied by the appearance of a new peak at 250 nm. The ^1H NMR spectrum of the product was indicative of two closely related compounds with the major signals (NCOMe, MeCH and SCH=CHN) being clearly twinned. These observations can be rationalised by aqueous hydrolysis of the azetidinone ring followed by the previously encountered migration of the double-bond to the Δ^1-position of the pyrroline ring. The resulting mixture of diastereoisomeric Δ^1-pyrrolines (**41**) has been characterised (as the barium salt) by Umezawa,[18] and a similar degradation product of asparenomycin A has also recently been described.[4]

The monomethyl ester of MM 4550 (**27**), which was prepared by alkylation (MeI, DMF) of the disodium salt (**4**) and showed the expected spectroscopic characteristics, failed to undergo oxidative degradation to a pyrrole in DMSO. This was a marked contrast to the corresponding MM 13902 ester (**12**) and has already been commented on. However, the utility of the DMSO-degradation reaction was demonstrated in confirming the structure of third olivanic acid, MM 17880.

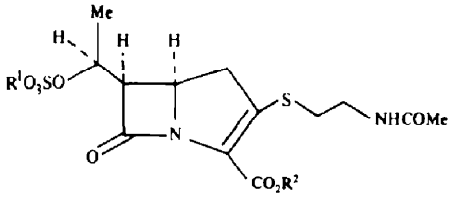
MM 17880 was isolated from *Streptomyces olivaceus* in the form of its disodium salt (**6**), the spectroscopic properties of which confirmed its close relationship to the earlier two metabolites. Significant differences were the lack of a short-wave absorption in the UV spectrum and the absence of the *trans*-disubstituted double bond protons in the ^1H NMR spectrum. The region δ 2.6–3.5 was complex but contained four protons in excess of those observed in the ^1H NMR spectra of **3** and **4**. On this basis, it was thought that MM 17880 was the dihydro-derivative of either MM 4550 or MM 13902. Alkylation of **6** with *p*-nitrobenzyl bromide and *p*-bromobenzyl bromide gave the mono esters (**42** and **43**), respectively, and analytical data for the former were consistent with the sulphide rather than the sulphoxide structure. On heating the ester (**43**) in dimethylsulphoxide (70°, 2 hr) the previously observed β-lactam degradation occurred to afford the pyrrole (**45**), which was characterised by its conversion [(i) CH_2N_2, (ii) DBU] to the less polar pyrrole[2] (**46**). Consequently, there was little doubt that MM 17880 was the dihydro-derivative of MM 13902 and possessed structure **5**.

Alkylation of the sulphate moiety of **42** with Meerwein's reagent afforded the MM 17880 diester (**44**) as a stable crystalline solid. Elimination of sodium hydrogen sulphate with anhydrous K_2CO_3-DMF occurred in a stereospecific manner to afford only the (*E*)-ethylidene derivative (**47**). Using the previously outlined argument, this result was consistent with the (8*S*)-configuration in MM 17880, the (5*R*,6*R*)-stereochemistry being inferred from the coupling ($J_{5,6}$ = 6 Hz) of the β-lactam protons in the ^1H NMR spectrum of **6**. It has subsequently been shown that all the olivanic acids (i.e. metabolites from *Streptomyces olivaceus*) which are chiral at C-8 possess the (*S*)-configuration at that position,[19] and in this respect they differ from thienamycin[3] (**48**).

Other aspects of the chemistry of the olivanic acids, including their conversion into thienamycin analogues, will be the subject of separate publications.



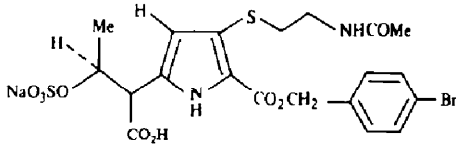
(41)



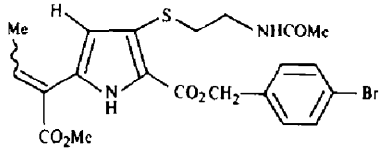
(42) $R^1 = Na$, $R^2 = PNB$

(43) $R^1 = Na$, $R^2 = CH_2$–Br

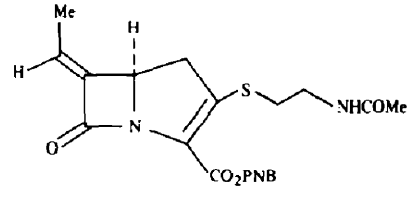
(44) $R^1 = Et$, $R^2 = PNB$



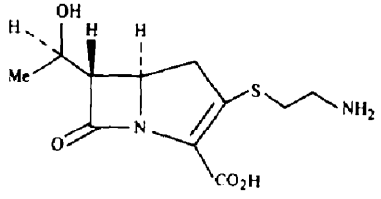
(45)



(46)



(47)



(48)

EXPERIMENTAL

M.ps were determined using a Kofler hot-stage apparatus and are uncorrected. UV spectra were recorded on a Pye–Unicam SP 800, SP 8000 or a Perkin–Elmer 554 spectrophotometer, and IR spectra on a Perkin–Elmer 157, 197 or 457 instrument. ^{1}H NMR spectra were obtained at 60 MHz with a Varian EM 360, at 90 MHz with a Perkin–Elmer R32 and at 250 MHz using Bruker WM 250 spectrometer. A Varian XL 100 machine was employed for ^{13}C NMR spectra. The internal standard for NMR spectra was TMS unless otherwise stated. Mass spectra were determined with an A.E.I. MS9 or VG 7070F instrument, except for field-desportion studies which were performed on a Varian CH5D mass spectrometer. Optical rotations were obtained by the use of a Perkin–Elmer 141 polarimeter and CD spectra were measured on a Cary-61 recording spectropolarimeter. HPLC was performed on a Waters Instruments 6000A machine using a C_{18} μ-Bondapak reverse-phase column eluting with pH 4.7 0.05M ammonium dihydrogen orthophosphate buffer at 2 ml min^{-1} and monitoring by UV absorption at λ 287 nm. The homogeneity of all compounds was established by TLC with Merck precoated silica-gel 60 F_{254} plates. Preparative chromatography was carried out on columns of Merck silica gel 60 (mixtures of 230–400 and finer than 230 mesh ASTM) using slightly increased pressure for elution. Organic solns were dried with $MgSO_4$ and solvents removed by evaporation under reduced pressure using a rotary evaporator. Organic solvents were either of Analar purity or were dried and/or distilled as necessary before use. Light petroleum refers to the fraction b.p. 60–80°.

Physical properties of the natural products. Compound MM 13902 (2) was isolated[7] from fermentation broth as its freeze-dried *disodium salt* (3); $[\alpha]_D^{20}$ (*c* 1 in H_2O) − 81°; ν_{max} (KBr) 1750, 1675, 1620 (broad) and 1270–1220 cm^{-1}; λ_{max} (H_2O) 307 (ϵ 15520) and 227 nm (14650); δ_H (D_2O) 1.47 (3H, d, *J* 6 Hz, MeCH), 2.00 (3H, s, MeCO), 2.94 (1H, dd, *J* 9.5 and 18 Hz, 4-CH_a), 3.29 (1H, dd, *J* 8.5 and 18 Hz, 4-CH_b), 3.78 (1H, dd, *J* 5.5 and 9 Hz, 6-CH), 4.25 (1H, approx. dt, *J* 5.5 and *ca* 9 Hz, 5-CH), 4.75 (1H, m, CHMe), 5.98 (1H, d, *J* 14 Hz, =CHS), and 7.07 (1H, d, *J* 14 Hz, =CHN) (MeCN internal standard at δ 2.00); δ_H (DMSO-d_6) *inter alia* 5.87 (1H, d, *J* 14 Hz, =CHS), 6.98 (1H, dd, *J* 14 and 10 Hz, =CHNH), and 10.25 (1H, broad d, *J* 10 Hz, NH); δ_C (D_2O) 19 (q, MeCH), 23 (q, MeCO), 37 (t, 4-C), 54 and 58 (each d, 5-C and 6-C), 74 (d, CHMe), 103 (d, =CHS), 128 (s, 3-C), 131 (d, =CHN), 144 (s, 2-C), 169 (s, CO), 172 (s, CO) and 178 (s, CO) (1,4-dioxan internal standard at δ 67.4); CD (*c* 0.04 in 0.01M pH 7 phosphate buffer) $[\theta]_{360}0$, $[\theta]_{339} - 0.27 \times 10^4$, $[\theta]_{326} - 0.41 \times 10^4$, $[\theta]_{292} - 0.22 \times 10^4$, $[\theta]_{270}0$, $[\theta]_{204} + 0.11 \times 10^4$, $[\theta]_{222} - 4.4 \times 10^4$, $[\theta]_{200}0$.

(5R,6R) - 3 - [(E) - 2 - *Acetamidoethenylsulphinyl*] - 6 - [(S) - 1 - *hydroxysulphonyloxyethyl*] - 7 - *oxo* - 1 - *azabicyclo*[3.2.0]*hept* - 2 - *ene* - 2 - *carboxylic acid* (MM 4550) (1). MM 4550 (1) was isolated[7] as its freeze-dried *disodium salt* (4); $[\alpha]_D^{20}$ (*c* 0.52 in H_2O) − 137°; ν_{max} (KBr) 1765, 1695, 1620 and 1260–1220 (broad) cm^{-1}; λ_{max} (H_2O) 287 (ϵ 12110)

and 240 nm (13560); δ_H (D_2O) 1.45 (3H, d, *J* 6.5 Hz, MeCH), 2.05 (3H, s, MeCO), 2.99 (1H, dd, *J* 9 and 18.5 Hz, 4-CH_a), 3.46 (1H, dd, *J* 10.5 and 18.5 Hz, 4-CH_b), 3.88 (1H, dd, *J* 6 and 9 Hz, 6-CH), 4.37 (1H, m, 5-CH), 4.97 (1H, m, CHMe), 6.34 (1H, d, *J* 14 Hz, =CHS), and 7.53 (1H, d, *J* 14 Hz, =CHN) (MeCN internal standard); δ_H (DMSO-d_6) *inter alia* 6.24 (1H, d, *J* 14 Hz, =CHS), 7.18 (1H, dd, *J* 14 and 11 Hz, =CHNH) and 10.65 (1H, broad d, *J* 11 Hz, NH); δ_C (D_2O) 19 (q, MeCH), 23 (q, MeCO), 29 (t, 4-C), 55 and 59 (each d, 5-C and 6-C), 74 (d, CHMe), 112 (d, =CHS), 139 (s, 3-C), 135 (d, =CHN), 140 (2-C), 166 (s, CO), 173 (s, CO) and 177 (s, CO) (1,4-dioxan internal standard); CD (*c* 0.04 in 0.01M pH 7 phosphate buffer) $[\theta]_{345}0$, $[\theta]_{289} - 3.2 \times 10^4$, $[\theta]_{261} - 3.6 \times 10^4$, $[\theta]_{247}0$, $[\theta]_{234} + 5.14 \times 10^4$, $[\theta]_{221}0$, $[\theta]_{210} - 2.06 \times 10^4$.

(5R,6R) - 3 - (2 - *Acetamidoethylthio*) - 6 - [(S) - 1 - *hydroxysulphonyloxyethyl*] - 7 - *oxo* - 1 - *azabicyclo*[3.2.0]*hept* - 2 - *ene* - 2 - *carboxylic acid* (MM 17880) (**5**). The freeze-dried *disodium salt* (**6**) of MM 17880[7] possesses the following characteristics; $[\alpha]_D^{20}$ (*c* 1.0 in H_2O) + 13.5°; ν_{max} (KBr) 1750, 1690–1650 and 1270–1220 cm^{-1}; λ_{max} (H_2O) 298 nm (ϵ 8410); δ_H (D_2O) 1.45 (3H, d, *J* 6 Hz, MeCH), 1.92 (3H, s, MeCO), 2.6–3.5 (6H, m, 4-CH_2 and SCH_2CH_2N), 3.79 (1H, dd, *J* 6 and 9.5 Hz, 6-CH), 4.26 (1H, m, 5-CH) and 4.77 (1H, m, CHMe) (MeCN internal standard); CD (*c* 0.01 in 0.01M pH 7 phosphate buffer) $[\theta]_{360}0$, $[\theta]_{326} - 0.15 \times 10^4$, $[\theta]_{312}0$, $[\theta]_{290}$ 0.44×10^4, $[\theta]_{250}0$, $[\theta]_{213} - 2.55 \times 10^4$, $[\theta]_{190}0$.

General procedure for the preparation of monoesters. The disodium salt (**3**, **4** or **6**) (0.01 mole) was stirred at room temp. with the appropriate alkyl halide (0.02 mole) in N,N-dimethylformamide (DMF) (5–10 ml) for 1.5–3 hr. The soln was evaporated almost to dryness and the residue was chromatographed employing a gradient elution of 0–40% EtOAc in $CHCl_3$. Fractions containing the product were combined and evaporated and residual EtOH was removed by the addition of toluene, EtOH or acetonitrile followed by further evaporation to afford an amorphous solid. Further purification could sometimes be effected by trituration with ether or by crystallisation, but where crystalline monoesters were obtained, m.ps are not quoted owing to decomposition before the melting temp. was attained.

Sodium salt of methyl (5R,6R) - 3 - [(E) - 2 - *acetamidoethenylthio*] - 6 - [(S) - 1 - *hydroxysulphonyloxyethyl*] - 7 - *oxo* - 1 - *azabicyclo*[3.2.0]*hept* - 2 - *ene* - 2 - *carboxylate* (**12**). From the disodium salt of MM 13902, **3** (1.1 g) and iodomethane (1.5 g) compound **12** was prepared as a white solid (0.68 g, 63%); λ_{max} (H_2O) 323 and 228 nm; ν_{max} (KBr) 1760, 1685, 1620 and 1280–1220 cm^{-1}; δ_H (DMSO-d_6) 1.33 (3H, d, *J* 6 Hz, MeCH), 1.92 (3H, s, MeCO), 2.92 (1H, dd, *J* 19 and 10 Hz, 4-CH_a), *ca* 3.5 (1H, m, 4-CH_b), 3.75 (4H, s + m, MeO and 6-CH), 4.20 (1H, m, 5-CH), 4.38 (1H, m, CHMe), 5.82 (1H, d, *J* 14 Hz, =CHS), 7.07 (1H, dd, *J* 14 and 11 Hz, =CHNH, collapses to d, *J* 14 Hz with D_2O) and 10.45 (1H, broad d, *J* 11 Hz, NH, exchanges with D_2O); δ_C (DMSO-d_6) 19.6 (q, MeCH), 22.5 (q, MeCO), 36.5 (t, 4-C), 51.6 (q, OMe), 53.3 and 58.3 (each d, 5-C and 6-C), 68.4 (d, CHMe), 97.2 (d, =CHS), 121.3 (s, =C<), 132.7 (d, =CHN), 151.7 (s, =C<), 161.2, 167.6 and 176.5 (each s, CO).

From the disodium salt **3** (0.97 g) and *p*-bromobenzyl bromide (1.7 g) was obtained the corresponding *mono*-p-*bromobenzyl ester* (**13**) as a white solid (0.73 g, 56%); λ_{max} (H_2O) 325 and 226 nm; ν_{max} (KBr) 1760, 1675, 1620 and 1280–1210 cm^{-1} [Found: (f.d. mass spectrum) *m/e* 480, 462 and 394 corresponding to (M–SO_3Na) + 1, M–HSO_4Na, and M–$C_4H_5SO_5Na$, respectively!.] A sample was crystallised from water–acetonitrile to afford needles; Found: C, 41.3; H, 3.7; N, 4.35; S, 10.65%. $C_{20}H_{20}N_2O_8S_2BrNa$ requires: C, 41.15; H, 3.4; N, 4.8; S, 11.0%.

MM 13902 disodium salt **3** (1.67 g) and *p*-nitrobenzyl bromide (2.48 g) afforded the *mono*-p-*nitrobenzyl ester* (**14**) as a pale-yellow solid (1.27 g, 60%); $[\alpha]_D^{20}$ (*c* 2 in H_2O) − 90°, λ_{max} (EtOH) 325 (ϵ 15400), 266 (15700), 225 sh and 220 nm (16700); ν_{max} (KBr) 1760, 1680, 1620 and 1280–1210 cm^{-1}; δ_H (DMSO-d_6) 1.36 (3H, d, *J* 6 Hz, MeCH), 1.92 (3H, s, MeCO), 2.96 (1H, dd, *J* 19 and 10 Hz, 4-CH_a), 3.52 (1H, dd, *J* 19 and 9 Hz, 4-CH_b), 3.67 (1H, dd, *J* 6 and 10.5 Hz, 6-CH), *ca* 4.20 (1H, m, 5-CH), 4.42 (1H, m, CHMe), 5.42 and 5.22 (each 1H, d, *J* 14 Hz, $CH_2C_6H_4$–NO_2), 5.80 (1H, d, *J* 14 Hz, =CHS), 7.04 (1H, dd, *J* 14 and 10.5 Hz, =CHNH, collapses to d, *J* 14 Hz on D_2O exchange), 7.63 and 8.17 (each 2H, d, *J* 8.5 Hz, C_6H_4–NO_2), and 10.38 (1H, d, *J* 10.5 Hz, NH, exchanges with D_2O); Found: (f.d. mass spectrum) *m/e* 447, 429 and 361 corresponding to (M–SO_3Na) + 1. M–HSO_4Na, and M–$C_4H_5SO_5Na$, respectively.[1] Some of the product crystallised from the column fractions and was recrystallised from ethanol to furnish fine needles; $[\alpha]_D^{20}$ (*c* 1 in H_2O) − 92° (*c* 0.5 in DMF) − 190°; λ_{max} (H_2O), 322 (ϵ 16990), 264 (16800) and 218 nm (17690). Found: C, 43.7; H, 3.75; N, 7.7; S, 11.6%. $C_{20}H_{20}N_3O_{10}S_2Na$ requires: C, 43.7; H, 3.7; N, 7.65; S, 11.7%.

From the disodium salt **3** (0.25 g) and *p*-bromophenacyl bromide (0.8 g) was obtained the *mono*-p-*bromophenacyl ester* (**15**). Some of the column fractions deposited crystals (64 mg); Found: C, 39.9; H, 3.65; N, 4.1; S, 10.1; Br, 12.55%. $C_{21}H_{20}N_2BrO_9S_2Na$. H_2O requires: C, 40.05; H, 3.5; N, 4.45; S, 10.15; Br, 12.7%. The remaining product was obtained as a solid (144 mg); λ_{max} (EtOH) 327 and 257 nm; ν_{max} (KBr) 1765, 1690, 1620 and 1280–1210 cm^{-1}.

Sodium salt of methyl (5R,6R) - 3 - [(E) - 2 - *acetamidoethenylsulphinyl*] - 6 - [(*S*) - 1 - *hydroxysulphonyloxyethyl*] - 7 - *oxo* - 1 - *azabicyclo*[3.2.0]*hept* - 2 - *ene* - 2 - *carboxylate* (**27**). From the disodium salt of MM 4550, **4** (0.504 g) and idomethane (1 ml), compound **27** was obtained (0.12 g, 23%). The product eluted off the column after NaI and was probably contaminated with a little of the latter. The ester was isolated as a white solid; λ_{max} (H_2O) 300 (ϵ 8900) and 243 nm (17200); ν_{max} (KBr) 1775, 1710, 1620 and 1260–1220 cm^{-1}; δ_H (D_2O) 1.49 (3H, d, *J* 6 Hz, MeCH), 2.10 (3H, s, MeCO), 3.0–4.1 (3H, m, 4-CH_2 and 6-CH), 3.87 (3H, s, MeO), 4.38 (1H, m, 5-CH), 4.95 (1H, m, CHMe), 6.37 (1H, d, *J* 14 Hz, =CHSO), 7.65 (1H, d, *J* 14 Hz, =CHN) [sodium 3-trimethylsilyltetradeuteriopropionate (DSS) internal standard].

Sodium salt of p - *nitrobenzyl* (5R,6R) - 3 - (2 - *acetamidoethylthio*) - 6 - [(S) - 1 - *hydroxysulphonyloxyethyl*] - 7 - *oxo* - 1 - *azabicyclo*[3.2.0]*hept* - 2 - *ene* - 2 - *carboxylate* (**42**). The disodium salt of MM 17880, **6** (2.00 g) and *p*-nitrobenzyl bromide (3.0 g) gave the title *mono*-p-*nitrobenzyl ester* (**42**). The product crystallised from the column fractions and was obtained by filtration and the mother liquors were evaporated to give a further quantity of ester as a white solid (0.931 g) after trituration with ether. The crystalline ester (0.245 g) possessed the following characteristics; $[\alpha]_D^{20}$ (*c* 2 in H_2O) + 19°; λ_{max} (H_2O) 318 (ϵ 14300) and 267 nm (12100); ν_{max} (KBr) 1765, 1695, 1655 and 1270–1220 cm^{-1}; δ_H (DMF-d_7) (250 MHz) 1.49 (3H, d, *J* 6.5Hz, MeCH), 1.92 (3H, s, MeCO), 3.06 (2H, m, CH_2S), 3.26 (1H, dd, *J* 10 and 19 Hz, 4-CH_a), 3.43 (2H, m, CH_2N), 3.77 (1H, dd, *J* 5.5 and 11 Hz, 6-CH), 3.97 (1H, dd, *J* 19 and 8.5 Hz, 4-CH_b), 4.35 (1H, m, 5-CH), 4.58 (1H, m, CHMe), 5.37 and 5.55 (each 1H, d, *J* 14 Hz, $CH_2C_6H_4$–NO_2), 7.84 and 8.30 (each 2H, d, *J* 9 Hz, C_6H_4–NO_2), and 8.20 (1H, broad t, *J* 11 Hz, NH); Found: C, 43.4; H, 3.85; N, 7.5%. $C_{20}H_{22}N_3O_{10}S_2Na$ requires: C, 43.55; H, 4.0; N, 7.6%.

The use of *p*-bromobenzyl bromide (0.75 g) with the disodium salt **6** (0.3 g) gave the corresponding mono-*p*-bromobenzyl ester (**43**) as a white solid (0.195 g; 49%); λ_{max} (H_2O) 320 nm; ν_{max} (KBr) 1765, 1690, 1635 and 1220 cm^{-1}; δ_H (DMSO-d_6) 1.37 (3H, d, *J* 6 Hz, MeCH), 1.81 (3H, s, MeCO), 2.8–3.6 (6H, m, 4-CH_2 and SCH_2CH_2N), 3.69 (1H, dd, *J* 6 and 11 Hz, 6-CH), 4.05–4.6 (2H, m, 5-CH and CHMe), 5.08 and 5.26 (each 1H, d, *J* 14 Hz, $CH_2C_6H_4$–Br), 7.36 and 7.57 (each 2H, d, *J* 8.5 Hz, C_6H_4–Br), and 8.10 (1H, broad, NH).

Benzyldimethyl - n - hexadecylammonium salt of p - *nitrobenzyl* (5R,6R) - 3 - [(E) - 2 - *acetamidoethenylthio*] - 6-

[(S) - 1 - *hydroxysulphonyloxyethyl*] - 7 - *oxo* - 1 - *azabicyclo*[3.2.0]*hept* - 2 - *ene* - 2 - *carboxylate* (**21**). A soln of the monosodium salt **14** (1.29 g) in water (30 ml) and a soln of benzyldimethyl-n-hexadecylammonium chloride (0.89 g) in $CHCl_3$ (30 ml) were vigorously shaken together in a separating funnel. A little brine was added to aid separation and the organic layer was collected and dried. Evaporation of the solvent afforded the title **21** as a yellow foam (1.85 g, 89%); ν_{max} ($CHCl_3$) 3280, 1780, 1700 and 1625 cm^{-1}.

Diesters of MM 13902 *and* MM 17880: p - *Nitrobenzyl* (5R,6R) - 3 - [(E) - 2 - *acetamidoethenylthio*] - 6 - [(S) - 1-*ethoxysulphonyloxyethyl*] - 7 - *oxo* - 1 - *aza* - *bicyclo*[3.2.0]*hept* - 2 - *ene* - 2 - *carboxylate* (**19**). The monoester **14** (0.21 g) was converted into **21** as previously described, and a soln of the product in CH_2Cl_2 (2 ml) was treated with a soln of triethyloxonium tetrafluoroborate (0.07 g) in CH_2Cl_2 (0.7 ml). After 30 min a further quantity of reagent (0.01 g in 0.1 ml solvent) was added and 5 min later the mixture was quickly chromatographed eluting with EtOAc–cyclohexane (4 : 1). Fractions containing the product were combined and evaporated, and the residual oil was crystallised from EtOAc–cyclohexane to afford the title *diester* **19** (61 mg, 29%); m.p. 125–128° (dec); $[\alpha]_D^{20}$ (*c* 1 in $CHCl_3$) – 184°; λ_{max} (EtOH) 325 (ϵ 19100), 265 (19600) and 219 nm (19000) ν_{max} (CH_2Cl_2) 1780, 1700 and 1620 cm^{-1}; δ_H ($CDCl_3$) 1.38 (3H, t, *J* 7 Hz, $MeCH_2$), 1.62 (3H, d, *J* 6 Hz, MeCH), 2.04 (3H, s, MeCO), 2.99 (1H, dd, *J* 19 and 10 Hz, 4-CH_a), 3.25 (1H, dd, *J* 19 and 9 Hz, 4-CH_b), 3.78 (1H, dd, *J* 5.5 and 10 Hz, 6-CH), 4.28 (2H, q, *J* 7 Hz, OCH_2Me), 4.98 (1H, m, CHMe), *ca* 5.2 (1H, m, 5-CH), 5.16 and 5.42 (each 1H, d, *J* 14 Hz, $CH_2C_6H_4$–NO_2), 5.78 (1H, d, *J* 13.5 Hz, =CHS), 7.20 (1H, dd, *J* 13.5 and 10 Hz, =CHNH), 7.53 and 8.11 (each 2H, d, *J* 9 Hz, C_6H_4–NO_2) and 8.26 (1H, d, *J* 10 Hz, NH); Found: C, 47.55; H, 4.55; N, 7.6%. $C_{22}H_{25}N_3O_{10}S_2$ requires: C, 47.55; H, 4.55; N, 7.55%.

A soln of **12** (0.1 g) in water (2 ml) was shaken with a soln of benzyldimethyl-n-hexadecylammonium chloride (0.093 g) in CH_2Cl_2 (2 ml). The organic layer was separated, dried, and evaporated before adding toluene and again removing the solvent by evaporation to eliminate any traces of water. The resulting ammonium salt **20** was dissolved in CH_2Cl_2 (2 ml) and the cooled (ice-bath) soln then stirred for 30 min with triethyloxonium tetrafluoroborate (55 mg). After evaporation of the solvent, the product was chromatographed using EtOAc as eluant. Fractions containing the product were combined and evaporated, before adding a little more ethyl acetate and removing a small quantity of insoluble impurity by filtration. Removal of the solvent then afforded the corresponding *methyl, ethyl-sulphate diester* **18** (50 mg, 49%) as a clear oil; ν_{max} (CH_2Cl_2) 1790, 1705, 1625 and 1200 cm^{-1}; δ_H ($CDCl_3$) 1.44 (3H, t, *J* 7 Hz, $MeCH_2$), 1.64 (3H, d, *J* 6 Hz, MeCH), 2.08 (3H, s, MeCO), 3.01 (1H, dd, *J* 18 and 9 Hz, 4-CHa), 3.23 (1H, dd, *J* 18 and 9 Hz, 4-CH_b), 3.84 (3H, s, MeO), *ca* 3.82 (1H, m, 6-CH), 4.35 (2H, q, *J* 7 Hz, OCH_2Me), 4.38 (1H, m, 5-CH), 5.03 (1H, m, CHMe), 5.85 (1H, d, *J* 14 Hz, =CHS), 7.30 (1H, dd, *J* 14 and 10 Hz, =CHNH), 8.18 (1H, d, *J* 10 Hz, NH). A sample of the product was crystallised from EtOAc–cyclohexane and recrystallised from EtOAc. X-ray analysis of the crystals furnished the structure shown in Fig. 1.[17]

By an analogous procedure the mono-*p*-nitrobenzyl ester of MM 17880, **42** (0.106 g) was converted into p-*nitrobenzyl* (5R,6R) - 3 - (2 - *acetamidoethylthio*) - 6 - [(S) - 1 - *ethoxysulphonyloxyethyl*] - 7 - *oxo* - 1 - *azabicyclo*[3.2.0]-*hept* - 2 - *ene* - 2 - *carboxylate* **44** (28 mg, 31%), which was crystallised from EtOH to give small needles; m.p. 126–128°; λ_{max} (EtOH) 318 (ϵ 13600) and 270 nm (11900); ν_{max} (KBr) 1765, 1690 and 1645 cm^{-1}; Found: C, 47.45; H, 5.05; N, 7.5%. $C_{22}H_{27}N_3O_{10}S_2$ requires: C, 47.4; H, 4.9; N, 7.55%.

p-*Bromobenzyl* (5R,6R) - 3 - [(E) - 2 - *acetamidoethenylthio*] - 6 - [(S) - 1 - *ethoxysulphonyloxyethyl*] - 7 - *oxo* - 1 - *azabicyclo*[3.2.0]*hept* - 2 - *ene* - 2 - *carboxylate* (**17**). The ester **13** (0.2 g) was suspended in CH_2Cl_2 (10 ml) and a soln of triethyloxonium tetrafluoroborate (65 mg) in CH_2Cl_2 (0.65 ml) was added with stirring. After 35 min at room temp. a further portion (10 mg) of alkylating agent was added, and 10 min after this the mixture was loaded onto a column of silica gel. Rapid elution with EtOAc–cyclohexane (4 : 1) gave the title *diester* **17** (0.092 g, 46%) which was crystallised from EtOAc–cyclohexane; m.p. 121–124° (dec); $[\alpha]_D^{20}$ (*c* 1 in $CHCl_3$) – 125°; λ_{max} (EtOH) 325 (ϵ 17340) and 227 nm (24300); ν_{max} (CH_2Cl_2) 1785, 1705, 1625 and 1195 cm^{-1}; δ_H ($CDCl_3$) 1.41 (3H, t, *J* 7 Hz, $MeCH_2$), 1.63 (3H, d, *J* 6 Hz, MeCH), 2.04 (3H, s, COMe), 2.98 (1H, dd, *J* 18.5 and 10 Hz, 4-CH_a), 3.24 (1H, dd, *J* 18.5 and 9 Hz, 4-CH_b), 3.76 (1H, dd, *J* 10 and 5.5 Hz, 6-CH), 4.25 (1H, m, 5-CH), 4.28 (2H, q, *J* 7 Hz, OCH_2Me), 4.95 (1H, m, CHMe), 5.07 and 5.24 (each 1H, d, *J* 13 Hz, $CH_2C_6H_4$–Br), 5.76 (1H, d, *J* 13.5 Hz, =CHS), *ca* 7.3 (1H, m, =CHNH), 7.22 and 7.41 (each 2H, d, *J* 9 Hz, C_6H_4–Br), and 7.98 (1H, d, *J* 10 Hz, NH); Found: C, 44.8; H, 4.4; N, 4.85%. $C_{22}H_{25}BrN_2O_8S_2$ requires: C, 44.8; H, 4.25; N, 4.75%.

Methyl (5R,6R) - 3 - [(E) - 2 - *acetamidoethenylthio*] - 6-[(S) - 1 - *methoxysulphonyloxyethyl*] - 7 - *oxo* - 1 - *azabicyclo*[3.2.0]*hept* - 2 - *ene* - 2 - *carboxylate* (**16**) The salt of MM 13902, **3** (0.3 g) was suspended in EtOH (26 ml) and water (3.5 ml) was added until dissolution was complete. To the cooled (0°) soln was added a cold soln of toluene-*p*-sulphonic acid (0.48 g) in EtOH (32 ml). After 5 seconds, excess etheral diazomethane was added and the soln stirred at 0° for 15 min. The solvents were removed by evaporation and the residual semi-solid was triturated with EtOAc. The crude ester **12**, contaminated with sodium toluene-*p*-sulphonate, was removed by filtration and the remaining soln rapidly chromatographed using EtOAc as eluant. The title *dimethyl ester* was obtained as a clear oil (40 mg, 14%); ν_{max} ($CHCl_3$) 3300 broad, 1780, 1700 and 1625 cm^{-1}; δ_H ($CDCl_3$) 1.45 (3H, d, *J* 6 Hz, MeCH) 2.07 (3H, s, MeCO), 3.12 (2H, m, 4-CH_2), *ca* 3.85 (1H, m, 6-CH), 3.82 and 3.96 (each 3H, s, OMe), 4.25 (1H, m, 5-CH), 5.0 (1H, m, CHMe), 5.82 (1H, d, *J* 13.5 Hz, =CHS), 7.27 (1H, dd, *J* 13.5 and 11 Hz, =CHNH) and 7.73 (1H, broad d, *J* 11 Hz, NH); Found: C, 43.3; H, 4.7; N, 6.2%; M^+, 420 (FD mass spectrum). $C_{15}H_{20}N_2O_8S_2$ requires: C, 42.85; H, 4.8; N, 6.65%; *M*, 420.

β-Lactam degradation reactions

Reactions of monoesters with dimethysulphoxide (DMSO) followed by diazomethane and 1,8-*diazabicyclo*-[5.4.0]*undec*-7-*ene* (DBU). The ester of MM 13902, **12** (0.3 g) was heated at 70° in DMSO-d_6 (0.5 ml) for 2 hr in an NMR tube. (The reaction also occurs if left for 3 days at room temp.). ^1H and ^{13}C NMR spectroscopy showed that complete rearrangement had occurred to give the monosodium salt of methyl 3 - [(*E*) - 2 - acetamidoethenylthio] - 5 - [(1R,2S) - 1 - carboxy - 2 - hydroxysulphonyloxyprop - 1 - yl]pyrrole - 2 - carboxylate (**22**); δ_H (DMSO-d_6) 1.24 (3H, d, *J* 6 Hz, MeCH), 1.93 (3H, s, MeCO), 3.72 (3H, s, MeO), 3.80 (1H, d, *J* 6 Hz, $CHCO_2H$), 4.68 (1H, overlapping qd, *J* 6 and 6 Hz, CHMe). 5.84 (1H, d, *J* 14 Hz, =CHS), 5.97 (1H, d, *J* 2.5 Hz, collapses to s on D_2O exchange, 4-CH), 7.06 (1H, dd, *J* 14 and 11 Hz, =CHNH, collapses to d, *J* 14 Hz on D_2O exchange), 10.35 (1H, d, *J* 11 Hz, NHCH= exchanges with D_2O) 11.53 (1H, broad, pyrrole NH, exchanges with D_2O), *ca* 11.5–13.5 (1H, very broad, acidic OH, exchanges with D_2O); δ_C (DMSO-d_6 + D_2O) 18.8 (q, MeCH), 22.5 (q, MeCO), 50.5 (d, $CHCO_2H$), 50.8 (q, OMe), 72.4 (d, CHMe), 100.5 (d, =CHS), 107.8 (d, pyrrole 4-CH), 115.4 (s, pyrrole-C), 125.9 (s, pyrrole-C), 130.0 (d, =CHN), 132.9 (s, pyrrole-C), 160.0, 167.3 and 171.2 (each s, CO).

Excess ethereal diazomethane was added to the soln and the solvents were then removed by evaporation. The residue was triturated with EtOAc and the washings rejected to leave a brown gum. This was dissolved in THF (10 ml) containing DMF (0.5 ml) and the soln was treated with DBU until no further ppt was formed. EtOAc was added and the solid removed by filtration before washing the

filtrate with dilute HCl aq, water and brine. Evaporation of the dried organic solution gave *methyl* 3 - (2 - *acetamidoethenylthio*) - 5 - (1 - *methoxycarbonylprop* - 1 - *enyl*)*pyrrole* - 2 - *carboxylate* **23** (45 mg) as a *ca* 5 : 1 mixture of (*E*)- and (*Z*)-isomers; λ_{max} (EtOH) 291 and 218 nm; ν_{max} ($CHCl_3$) 3400, 1700 broad, and 1625 cm^{-1}; δ_H ($CDCl_3$) (*E*)-isomer, 1.98 (3H, d, *J* 7 Hz, MeCH=), 2.04 (3H, s, MeCO), 3.77 and 3.83 (each 3H, s, MeO), 5.90 (1H, d, *J* 13 Hz, =CHS), 6.14 (1H, d, *J* 3 Hz, pyrrole 4-CH), 7.15 (1H, q, *J* 7 Hz, =CHMe), 7.25 (1H, dd, *J* 13 and 10 Hz, =CHNH), 8.06 (1H, broad d, *J* 10 Hz, NH) and 10.17 (1H, broad, pyrrole-NH), (*Z*)-isomer shows a signal at δ 6.65 (q, *J* 8 Hz, =CHMe); Found: M^+, 338.0934. $C_{15}H_{18}N_2O_5S$ requires: 338.0936.

A soln of the *p*-bromobenzyl ester of MM 17880, **43** (50 mg) in DMSO-d_6 at 70° for 2 hr similarly gave the monosodium salt of **45**; δ_H (DMSO-d_6) 1.28 (3H, d, *J* 6 Hz, MeCH), 1.80 (3H, s, MeCO), 2.88 (2H, t, *J* 7 Hz, CH_2S), 3.22 (2H, m, CH_2N), 3.80 (1H, d, *J* 8 Hz, $CHCO_2$), 4.69 (1H, m, CHMe), 5.25 (2H, s, $CH_2C_6H_4$–Br), 6.21 (2H, d, *J* 2 Hz, pyrrole 4-CH), 7.50 (4H, m, C_6H_4–Br), 8.07 (1H, broad, NH) and 11.60 (1H, broad, NH). Excess ethereal diazomethane was added followed by DBU (0.1 ml) and after 15 min at room temp. the soln was extracted with EtOAc and washed with dilute HCl, water and brine. Evaporation of the dried organic soln gave a residue which was chromatographed using EtOAc as eluant to afford *p-bromobenzyl* 3 - (2 - *acetamidoethylthio*) - 5 - (1 - *methoxycarbonylprop* - 1 - *enyl*)*pyrrole* - 2 - *carboxylate* **46** (8 mg) [Mainly (*E*)-isomer]; ν_{max} ($CHCl_3$) 1700 and 1670 cm^{-1}; δ_H ($CDCl_3$), (*E*)-isomer, 1.90 (3H, s, MeCO), 2.05 (3H, d, *J* 8 Hz, MeCH=), 2.97 (2H, t, *J* 8 Hz, CH_2S), 3.41 (2H, m, CH_2N), 3.78 (3H, s, MeO), 5.27 (2H, s, $CH_2C_6H_4$–Br), 6.12 (1H, br, NH), 6.38 (1H, d, *J* 3 Hz, pyrrole 4-CH), 7.05–7.55 (5H, m, =CHMe and C_6H_4–Br), and 10.25 (1H, broad, NH); Found: M^+, 494.0515. $C_{21}H_{23}N_2O_5SBr$ requires: 494.0515.

Methyl 5 - (1 - *ethoxycarbonylprop* - 1 - *yl*)*pyrrole* - 2 - *carboxylate* (**24**). A soln of **23** (45 mg) in EtOH (1 ml) was stirred at room temp. with freshly prepared W7 Raney Ni[20] (*ca* 1 ml) for 30 min. The mixture was filtered and, after evaporation of the solvent, the product was chromatographed using 50% light petroleum–EtOAc to elute. The title **24** was isolated as an oil (14 mg); ν_{max} ($CHCl_3$) 1720 and 1700 cm^{-1}; δ_H ($CDCl_3$) 0.94 (3H, t, *J* 7 Hz, $MeCH_2CH$), 1.29 (3H, t, *J* 7 Hz, $MeCH_2O$), 1.91 (2H, m, $MeCH_2CH$), 3.60 (1H, t, *J* 8 Hz, CH_2CH), 3.85 (3H, s, MeO), 4.20 (2H, q, *J* 7 Hz, OCH_2Me), 6.09 (1H, m, pyrrole 4-CH), 6.83 (1H, m, pyrrole 3-CH) and 9.45 (1H, broad, NH); Found: M^+, 239 ($C_{12}H_{17}NO_4$).

Reaction of the monomethyl ester (**12**) *with diethylamine and methanol.* A soln of **12** (0.25 g) in MeOH (10 ml) was stirred with Et_2NH (0.128 g) at room temp. for 20 hr. The solid (17 mg) formed in the reaction was filtered off, and identified as Na_2SO_4 by comparison of its IR spectrum with an authentic sample. The filtrate was evaporated and the product was chromatographed using chloroform as eluant to afford *methyl* 3 - *hydroxy* - 5 - (1 - *methoxycarbonylprop* - 1 - *enyl*)*pyrrole* - 2 - *carboxylate* **28** (30 mg, 21%) as a mixture (*ca* 7 : 1) *of* (*E*)- and (*Z*)-isomers; λ_{max} (EtOH) 291 nm; ν_{max} ($CHCl_3$) 1700, 1665 and 1570 cm^{-1}; δ_H ($CDCl_3$), *E*-isomer, 2.02 (3H, d, *J* 7 Hz, MeCH=), 3.78 and 3.84 (each 3H, s, MeO), 5.92 (1H, d, *J* 3 Hz, pyrrole 4-CH), 7.15 (1H, q, *J* 7 Hz, =CHMe), 7.40 (1H, broad, s, OH) and 9.50 (1H, broad, NH), (*Z*)-isomer similar to (*E*)-isomer except 2.08 (3H, d, *J* 7 Hz, MeCH=), 5.88 (1H, d, *J* 3 Hz, pyrrole 4-CH) and 6.65 (1H, q, *J* 7 Hz, =CHMe); Found: M^+, 239.0795. $C_{11}H_{13}NO_5$ requires: 239.0791.

Further elution with 25% EtOH–$CHCl_3$ gave an amorphous solid (137 mg, 51%) which was considered to be the Na-salt of a single C-3 isomer of **29**; λ_{max} (H_2O) 262 and 227 nm; ν_{max} (KBr) 1725, 1670 and 1630 cm^{-1}; δ_H (D_2O) 1.46 (3H, d, *J* 7 Hz, MeCH), *ca* 1.6–2.1 (1H, m, pyrroline 3-CH), *ca* 2.6–3.4 (2H, m, pyrroline 4-CH_2), 3.73 and 3.91 (each 3H, s, MeO), 4.40 (1H, m, pyrroline 5-CH), 4.85 (1H, m, CHMe), 5.65 (1H, d, *J* 13.5 Hz, =CHS), and 7.07 (1H, d, *J* 13.5 Hz, =CHN) (internal reference HOD at δ 4.68).

The pyrroline derivative was also obtained as a mixture of C-3 isomers by the following method. The ester **12** (0.3 g) was treated with NaOMe (39 mg, 1 mol equiv) in MeOH (10 ml) for 16 hr. The mixture was filtered and the filtrate evaporated before chromatographing the residue employing a gradient elution of 0–25% EtOH in $CHCl_3$. The first-eluted product was **28** (10 mg, 6%) followed by the diastereoisomeric (*ca* 2 : 1) pyrroline **29** (0.196 g, 60%); ν_{max} (KBr) 1725, 1670 and 1630 cm^{-1}; δ_H (DMSO-d_6) 1.28 and 1.30 (3H, each d, *J* 7 Hz, MeCH), *ca* 1.4–1.9 (1H, m, pyrroline 3-CH), 1.92 and 1.95 (3H, each s, MeCO), 3.58 and 3.80 (each 3H, s, MeO), 4.1–4.85 (3H, m, pyrroline 4-CH and 5-CH, and CHMe), 5.42 and 5.55 (1H, each d, *J* 14 Hz, =CHS), 7.0 (1H, m, consisting of dd, *J* 14 and 11 Hz, for each isomer) and 10.21 (1H, d, *J* 11 Hz, NH).

Hydrolysis of MM 4550. A soln of the disodium salt of MM 4550 **4** (40 mg) was allowed to stand in D_2O (0.4 ml) for 2 days at room temp. NMR showed that hydrolysis was complete. The soln was evaporated and the residue triturated with ether to afford a solid. The material was allowed to stand overnight in MeOH but no significant change occurred (TLC), so the soln was evaporated to dryness and the residue chromatographed on cellulose eluting with i-PrOH–n-BuOH–water (7 : 7 : 6). Later fractions yielded the disodium salt of **41**[18] (two C-3 diastereoisomers) (7 mg); λ_{max} (H_2O) 248 nm; δ (D_2O) *inter alia* 1.48 and 1.49 (3H, each d, *J ca* 6 Hz, MeCH), 2.10 and 2.12 (3H, each s, MeCO), 5.95 and 6.13 (1H, each d, *J* 14 Hz, =CHS) and 7.43 and 7.54 (1H, each d, *J* 14 Hz, =CHN) (DSS internal standard). The hydrolysis product (**41**) was also obtained from the final stage of purification[7] of MM 4550.

Oxidation of MM 13902 *with* m-*chloroperbenzoic acid.* A soln of MM 13902 disodium salt **3** (0.3 g) in water (10 ml) was stirred with solid *m*-chloroperbenzoic acid (0.17 g). A white ppt of *m*-chlorobenzoic acid was rapidly formed, and after 10 min was removed by filtration. The soln was neutralised to pH 7 with $NaHCO_3$ aq and then chromatographed on QAE Sephadex A25, eluting with 5% NaCl in 0.05M pH 7 phosphate buffer soln. Fractions containing the product (monitored by UV spectroscopy) were combined and concentrated to small volume. The resulting soln was then desalted on Amberlite XAD-4 using water to elute, and then freeze-dried to afford a mixture of **37** and **40** (1.2 : 1) as a white solid (0.176 g, 57%); λ_{max} (H_2O) 287 and 237 nm; ν_{max} (KBr) 1765, 1690, 1620 broad and 1270–1210 cm^{-1}; δ_H (D_2O) 1.42 (3H, d, *J* 6 Hz, MeCH), 2.02 (3H, s, MeCO) 2.80–3.55 (2H, m, ABX system for 4-CH_2), 3.85 (1H, m, 6-CH), 4.40 (1H, m, 5-CH), 4.80 (1H, m, CHMe), 6.20 and 6.25 (1H, each d, *J* 14 Hz, =CHS for minor and major isomers), and 7.45 (1H, d, *J* 14 Hz, =CHN) (HOD internal ref. at δ 4.55). HPLC and a comparison of ^{1}H NMR spectra revealed that the major diastereoisomer was identical with MM 4550 disodium salt.

Ethylidene derivatives

Methyl (5R) - 3 - [(E) - 2 - *acetamidoethenylthio*] - 6 - (E)-*ethylidene* - 7 - *oxo* - 1 - *azabicyclo*[3.2.0]*hept* - 2 - *ene* - 2 - *carboxylate* (**31**) *and the* 6 - (Z) - *isomer* (**33**). A soln of **12** (0.37 g) in DMF (6 ml) was stirred at room temp. with anhyd K_2CO_3 (0.56 g). After 20 hr, the solvent was removed by evaporation and the product partitioned between $CHCl_3$ and water. The dried organic layer was evaporated and the residue fractionated by chromatography. Elution with 20% light-petroleum–EtOAc afforded *methyl* (2S,5R) - 3 - [(E) - 2 - *acetamidoethenylthio*] - 6 - *ethylidene* - 7 - *oxo* - 1 - *azabicyclo*[3.2.0]*hept* - 3 - *ene* - 2 - *carboxylate* **35** (gum, 11 mg, 4%) as a mixture (*ca* 3 : 1) of (*E*)- and (*Z*)-isomers; ν_{max} ($CHCl_3$) 1760 broad, 1700 and 1625 cm^{-1}; δ_H ($CDCl_3$), (*E*)-isomer, 1.79 (3H, d, *J* 8 Hz, MeCH=), 2.03 (3H, s, MeCO), 3.72 (3H, s, OMe), *ca* 5.0 (2H, m, 2-CH and 5-CH), 5.65 (1H, d, *J* 14 Hz, =CHS), 5.75 (1H, d, *J* 5 Hz, 4-CH), 6.20 (1H, q, *J* 8 Hz, =CHMe), and 7.64 (1H, broad d, *J* 10 Hz,

NH), (*Z*)-isomer shows signals at *ca* 1.99 (d, MeCH=) and *ca* 5.8 (q, CHMe); Found: M^+, 308.0832. $C_{16}H_{14}N_2O_4S$ requires: 308.0831.

Further elution with EtOAc gave the title *ethylidene* as a mixture (*ca* 3 : 1) of the (*E*)- and (*Z*)-isomers (**31** and **33**) in the form of a pale yellow foam (0.16 g, 60%); ν_{max} ($CHCl_3$) 1770, 1700 and 1625 cm^{-1}; Found: M^+, 308.0829. $C_{14}H_{16}N_2O_4S$ requires: 308.0831.

Further chromatography of the product (0.15 g) using 0–4% EtOH–$CHCl_3$ to elute gave early fractions containing the pure (*Z*)-isomer **33** (20 mg); δ ($CDCl_3$) 2.03 (3H, d, MeCH=), 2.05 (3H, s, MeCO), 3.04 (2H, broad d, *J* 8 Hz, 4-CH_2), 3.80 (3H, s, MeO), 4.62 (1H, broad t, *J* 8 Hz, 5-CH), 5.90 [2H, m, consisting of (d, *J* 14 Hz, =CHS) and (q, =CHMe)], 7.17 (1H, dd, *J* 14 and 10.5 Hz, =CHNH) and 8.25 (1H, broad d, *J* 10.5 Hz, NH). The main bulk of product contained both isomers, but later fractions were combined to give the (*E*)-isomer **31** (28 mg); δ_H ($CDCl_3$) 1.81 (3H, d, *J* 7.5 Hz, MeCH), 2.05 (3H, s, MeCO), 2.93 (1H, dd, *J* 18 and 8.5 Hz, 4-CHa), 3.17 (1H, dd, *J* 18 and 10 Hz, 4-CH_b), 3.80 (3H, s, MeO), 4.70 (1H, broad t, *J ca* 9 Hz, 5-CH), 5.87 (1H, d, *J* 14 Hz, =CHS), 6.36 (1H, dq, *J* 7.5 and 1 Hz, =CHMe), 7.16 (1H, dd, *J* 14 and 10 Hz, =CHN) and 8.23 (1H, broad d, *J* 10 Hz, NH).

The (*E*)-isomer **31** alone was prepared by the following method; a soln of the diester **18** (42 mg) in EtOH (2 ml) was stirred with anhyd KOAc (50 mg). After 90 min the solvent was evaporated and the product partitioned between EtOAc and water. The organic phase was washed with brine and dried, followed by evaporation of the solvent and purification of the product by chromatography using EtOAc as eluant. The (*E*)-ethylidene **31** was obtained as a foam (14 mg, 46%), ν_{max} (CH_2Cl_2) 1775, 1700 and 1625 cm^{-1}.

p - *Nitrobenzyl* (5R) - 3 - [(E) - 2 - *acetamidoethenylthio*] - 6 - *ethylidene* - 7 - *oxo* - 1 - *azabicyclo*[3.2.0]*hept* - 2 - *ene* - 2 - *carboxylate* (**32**) *and the* 6 - (Z) - *isomer* (**34**). A soln of **21** (1.85 g) in CH_2Cl_2 (50 ml) was stirred with DBU at −10° for 3.5 hr. The soln was washed (×3) with dilute brine, dried and evaporated to give a crude product which was chromatographed. Elution with 20% light petroleum–EtOAc gave p - *nitrobenzyl* (2S,5R) - 3 - [(E) - 2 - *acetamidoethenylthio*] - 6 - *ethylidene* - 7 - *oxo* - 1 - *azabicyclo*[3.2.0]*hept* - 3 - *ene* - 2 - *carboxylate* (**36**) (46 mg, 6%) (Ratio of (*E*)- and (*Z*)-isomers = *ca* 1:1.3); ν_{max} ($CHCl_3$) 1760, 1700 and 1625 cm^{-1}; δ ($CDCl_3$), (*E*)-isomer, 1.80 (3H, d, *J* 7 Hz, MeCH=), 2.03 (3H, s, MeCO), *ca* 5.0 (2H, m, 2-CH and 5-CH), 5.22 (2H, s, $CH_2C_6H_4$–NO_2), 5.62 (1H, d, *J* 14 Hz, =CHS), 5.75 (1H, m, 4-CH), 6.21 (1H, qd, *J* 7 and 1 Hz, =CHMe), 7.13 (1H, dd, *J* 14 and 11 Hz, =CHNH), 7.45 and 8.14 (each 2H, d, *J* 8.5 Hz, C_6H_4–NO_2) and 7.6 (1H, broad d, *J* 11 Hz, NH), (*Z*)-isomer shows signals at *ca* 2.0 (d, MeCH) and *ca* 5.75 (q, =CHMe); Found: M^+, 429.0991. $C_{20}H_{19}N_3O_6S$ requires: 429.0994.

Elution with EtOAc gave the title *ethylidene* as a mixture (*ca* 1 : 1.3) of (*E*)-isomer **32** and (*Z*)-isomer **34**, obtained as a pale yellow solid (0.46 g, 57%) after trituration with ether; ν_{max} (KBr) 1760, 1690 and 1620 cm^{-1}; λ_{max} (EtOH) 340 sh (ϵ 11000), 310 (13400) and 268 nm (17400); δ (DMF-d_7) 1.82 and 2.00 [3H, each d, *J* 7 Hz, MeCH for (*E*)- and (*Z*)-isomer, respectively], *ca* 1.98 (3H, s, MeCO), *ca* 3.2 (2H, m, 4-CH_2), 4.60–5.00 (1H, m, 5-CH), 5.31 and 5.54 (each 1H, d, *J* 14 Hz, $CH_2C_6H_4$–NO_2), 5.95 (1H, d, *J* 13.5 Hz, =CHS), 6.12 [dq, *J* 0.5 and 7 Hz, =CHMe for (*Z*)-isomer], 6.38 [dq, *J* 1 and 7 Hz, =CHMe for (*E*)-isomer], 7.15 (1H, dd, *J* 13.5 and 10.5 Hz, =CHNH), 7.77 and 8.22 (each 2H, d, *J* 9 Hz, C_6H_4–NO_2) and 10.36 (1H, d, *J* 10.5 Hz, NH); Found: C, 55.75; H, 4.45; N, 9.7%, M^+, 429.0999. $C_{20}H_{19}N_3O_6S$ requires: C, 55.95; H, 4.45; N, 9.8%, *M*, 429.0994.

The pure (*E*)-isomer **32** was prepared by the following method; the MM 13902 diester **19** (0.109 g) was stirred with K_2CO_3 (0.1 g) in DMF (2 ml) for 90 min. After evaporation of the solvent, the residue was partitioned between EtOAc and water, and the organic layer was washed with brine, and dried. Chromatography of the product afforded the (E)-*ethylidene* **32**, (60 mg, 70%), which was recrystallised from EtOAc–hexane; m.p. 163–166°, $[\alpha]_D^{20}$ (*c* 0.5 in DMF) − 64°; λ_{max} (EtOH) 310 (ϵ 11880), 263 (15550) and 217 nm (23650); ν_{max} (KBr) 1760, 1695 and 1620 cm^{-1}; δ_H (DMF-d_7) 1.82 (3H, d, *J* 7 Hz, MeCH), 1.98 (3H, s, MeCO), 3.0–3.5 (2H, m, 4-CH_2), 4.83 (1H, broad t, *J* 9 Hz, 5-CH), 5.30 and 5.54 (each 1H, d, *J* 14 Hz, $CH_2C_6H_4$–NO_2), 5.96 (1H, d, *J* 13.5 Hz, =CHS), 6.38 (1H, dq, *J* 1 and 7 Hz, =CHMe), 7.16 (1H, dd, *J* 13.5 and 10 Hz, =CHNH), 7.77 and 8.21 (each 2H, d, *J* 9 Hz, C_6H_4–NO_2); Found: C, 55.75; H, 4.6; N, 9.65%; M^+, 429.0995. $C_{20}H_{19}N_3O_6S$ requires: C, 55.95, H, 4.45, N, 9.8%; M^+; 429.0994.

p - *Nitrobenzyl* (5R) - 3 - (2 - *acetamidoethylthio*) - 6 - (E) - *ethylidene* - 7 - *oxo* - 1 - *azabicyclo*[3.2.0]*hept* - 2 - *ene* - 2 - *carboxylate* (**47**). The ester **42** (1 g) was converted into **44** as already described, but without purification the crude product was dissolved in DMF (10 ml) and stirred with K_2CO_3 (750 mg) in the presence of a few 3A molecular sieves. After 25 min, work-up as in the previous experiment gave a residue which was chromatographed using a gradient elution of 0–15% EtOH–EtOAc. The (E)-*ethylidene* **47** was obtained as a solid (333 mg, 43% from **42**) by trituration with ether and was recrystallised from EtOAc–EtOH; m.p. 188–190°; λ_{max} (EtOH) 337 sh (ϵ 7400), 299 (11100) and 267 nm (11300); ν_{max} (KBr) 1775, 1700 and 1630 cm^{-1}; δ (DMF-d_7), 1.88 (3H, s, MeCO), 1.86 (3H, d, *J* 7 Hz, MeCH=), *ca* 3.0–3.75 (6H, m, 4-CH_2 and SCH_2CH_2N), 4.91 (1H, broad t, *J* 9 Hz, 5-CH), 5.34 and 5.58 (each 1H, d, *J* 14 Hz, $CH_2C_6H_4$–NO_2), 6.44 (1H, qd, *J* 7 and 1 Hz, =CHMe), 7.83 and 8.28 (each 2H, d, *J* 9 Hz, C_6H_4–NO_2), and 8.10 (1H, br, NH, exchanges with D_2O); Found: C, 55.65; H, 4.75; N, 9.65%, M^+, 431.1177. $C_{20}H_{21}N_3O_6S$ requires: C, 55.7; H, 4.85; N, 9.75%, *M*, 431.1148.

Acknowledgements—We would like to thank Dr. M. Scopes of Westfield College, University of London for the CD spectra, Prof. A. H. Jackson and Dr. D. E. Games of University College, Cardiff for the FD mass spectra, Dr. J. W. Emsley of Southampton University for some of the ^{13}C NMR spectra and Prof. T. J. King† of the University of Nottingham for the X-ray structure. We would also like to acknowledge Mr. J. W. Tyler, Mr. A. Cutmore and Mr. R. Edser† for recording IR and NMR spectra, and our other colleagues at Brockham Park for helpful discussions.

†Since this manuscript was submitted, the authors have reported the sad deaths of Prof. T. J. King and Mr. R. Edser.

REFERENCES

[1]A. G. Brown, D. F. Corbett, A. J. Eglington and T. T. Howarth, *J. Chem. Soc.* Chem. Commun. 523 (1977).
[2]D. F. Corbett, A. J. Eglington and T. T. Howarth, *Ibid.* Chem. Commun. 953 (1977).
[3]G. Albers-Schönberg, B. H. Arison, O. D. Hensens, J. Hirshfield, K. Hoogsteen, E. A. Kaczka, R. E. Rhodes, J. S. Kahan, F. M. Kahan, R. W. Ratcliffe, E. Walton, L. J. Ruswinkle, R. B. Morin and B. G. Christensen, *J. Am. Chem. Soc.* **100**, 6491 (1978).
[4]S. Harada, S. Shinagawa, Y. Nozaki, M. Asai and T. Kishi, *J. Antibiotics* **33**, 1425 (1980); M. Nakayama, A. Iwasaki, S. Kimera, T. Mizoguchi, S. Tanabe, A. Murakami, I. Watanabe, M. Okuchi, H. Itoh, Y. Saino, F. Kobayashi and T. Mori, *Ibid.* **33**, 1389 (1980).
[5]N. Tsuji, K. Nagashima, M. Kobayashi, J. Shoji, T. Kato, Y. Terui, H. Nakai and M. Shiro, *Ibid.* **35**, 24 (1982).
[6]K. Yamamoto, T. Yoshioka, Y. Kato, N. Shibamoto, K. Okamura, Y. Shimauchi and T. Ishikura, *Ibid.* **33**, 796 (1980).
[7]J. D. Hood, S. J. Box and M. S. Verrall, *Ibid.* **32**, 295 (1979).
[8]S. J. Box, J. D. Hood and S. R. Spear, *Ibid.* **32**, 1239 (1979).

[9]A. G. Brown, D. F. Corbett, A. J. Eglington and T. T. Howarth, *Ibid.* **32**, 961 (1979).
[10]U.K. Pat. Application No. 8021730, 1980. See also S. J. Box, D. F. Corbett, K. G. Robins, S. R. Spear and M. S. Verrall, *J. Antibiotics* **35**, 1394 (1982).
[11]R. A. Jones and G. P. Bean, *The Chemistry of Pyrroles*, p. 472, and refs. therein. Academic Press, London (1977).
[12]M. C. Kloetzel, J. L. Pinkus and R. M. Washburn, *J. Am. Chem. Soc.* **79**, 4222 (1957).
[13]A. G. Brown, D. F. Corbett and T. T. Howarth, *J. Chem. Soc.* Chem. Commun. 359 (1977).
[14]J. H. Bateson, R. I. Hickling, P. M. Roberts, T. C. Smale and R. Southgate, *Ibid.* Chem. Commun. 1084 (1980).
[15]D. H. Shih and R. W. Ratcliffe, *J. Med. Chem.* **24**, 639 (1981).
[16]D. F. Corbett, *J. Chem. Soc.* Chem. Commun. 803 (1981).
[17]Performed by T. J. King, University of Nottingham.
[18]K. Maeda, S. Takahashi, M. Sezaki, K. Iinuma, H. Naganawa, S. Kondo, M. Ohno and H. Umezawa, *J. Antibiotics* **30**, 770 (1977).
[19]D. F. Corbett and A. J. Eglington, *J. Chem. Soc.* Chem. Commun. 1083 (1980).
[20]R. L. Augustine, *Catalytic Hydrogenation*, p. 148. Edward Arnold, London and Marcel Dekker, New York (1965).