Structures of Olivanic Acid Derivatives† MM 4550 and MM 13902; Two New, Fused β-Lactams isolated from *Streptomyces olivaceus*

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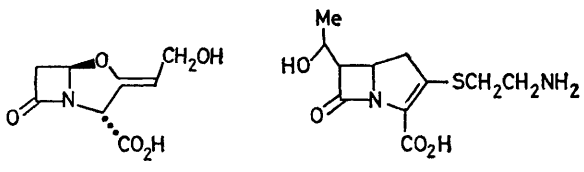
Summary The structures of two new, naturally occurring β-lactams are proposed on the basis of spectroscopic properties, and their interconversion.

RECENT reports have described the structures of clavulanic acid (**1**)[1] and thienamycin (**2**),[2] two naturally occurring β-lactams with novel, bicyclic ring systems. We now describe the spectroscopic evidence to support structures (**3**) and (**8**) for MM 13902 and MM 4550, two new β-lactam antibiotics isolated from *Streptomyces olivaceus*.[3]

The derivative MM 13902 was isolated as the freeze-dried disodium salt (**4**),‡ $C_{13}H_{14}N_2Na_2O_8S_2$, which had the following properties: $[\alpha]_D^{20}$ −81° (*c* 1·0 in H_2O); ν_{max} (KBr) 1750 (β-lactam CO), 1675 (amide CO), 1620br (CO_2^-), and 1220—1270br (OSO_3^-) cm^{-1}; λ_{max} (H_2O) 307 (ε 15,520) and 227 (14,650) nm. The ^1H n.m.r. spectrum in D_2O (rel. to internal standard MeCN, δ 2·00) revealed thirteen of the protons; the amide proton exchanged under these conditions.



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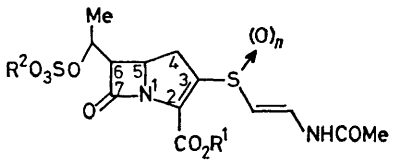
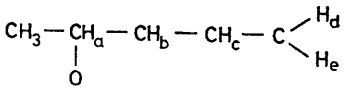


(3); $R^1 = R^2 = H$, $n = 0$ (8); $R^1 = R^2 = H$, $n = 1$
(4); $R^1 = R^2 = Na$, $n = 0$ (9); $R^1 = R^2 = Na$, $n = 1$
(5); $R^1 = Me$, $R^2 = Na$, $n = 0$ (10); $R^1 = R^2 = H$, $n = 2$

† We shall refer to structure (A) (see end of text) as olivanic acid.

‡ The empirical formula was obtained by elemental analysis of several crystalline monoester derivatives and mass spectral studies on the dimethyl ester.

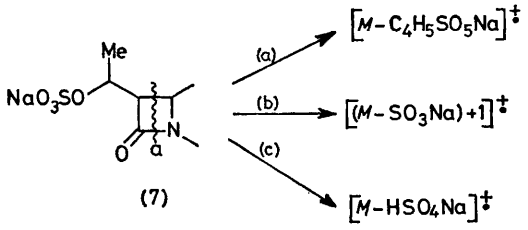
Eight of the protons appeared as part-structure (**6**) [δ 4·75 {H_a, J(Me-H_a) 6·0, J(H_a-H_b) 9·0 Hz}, 3·78 {H_b, J(H_b-H_c) 5·5 Hz}, 4·25 {H_c, J(H_c-H_d) 8·5, J(H_c-H_e) 9·5 Hz}, 3·29 {H_d, J(H_d-H_e) 18·0 Hz}, 2·94 (H_e), and 1·47 (Me)], in which substitution of the oxygen atom was inferred from the chemical shifts of the methyl- and H_a-protons. The observed coupling between H_b and H_c (J 5·5 Hz) was



(6)

reminiscent of the *cis*-coupling observed in the spectra of other β-lactams[4] and indicated the location of the azetidinone ring. The *trans*-olefinic protons were at δ 5·98 (d, J 14 Hz) and 7·07 (d, J 14 Hz) and the COMe protons at δ 2·00. The ^{1}H n.m.r. spectrum in $(CD_3)_2SO$ revealed the amide proton as part of the acylenamine system, –CONHCH=CH– [δ 5·87 (1H, d, J 14 Hz, =CH), 6·98 (1H, dd, J 14 and 10 Hz, NHC*H*=), and 10·25 (1H, br d, J 10 Hz, NH)]. The ^{13}C n.m.r. spectrum confirmed the above assignments (Table) and revealed the tetrasubstituted double bond.

Alkylation of (**4**) with methyl iodide in dimethylformamide gave the monoester (**5**) whose spectral data were in accord with the proposed structure and in which the methoxycarbonyl absorption appeared at 1685 cm^{-1}, indicating an $\alpha\beta$-unsaturated ester. The u.v. spectrum of (**5**) showed the long-wavelength absorption at 323 nm, precluding attachment of the ester group on the *trans*-disubstituted double bond since the resulting chromophore (–CONH.CH=CHCO$_2$Me) would be expected to absorb at lower wavelength.[5] Consequently the ester group was attached to the tetrasubstituted double bond.



(7)

SCHEME

Evidence for part structure (**7**) was obtained from the field-desorption mass spectra (obtained on a Varian CH5D mass spectrometer by A. H. Jackson and D. E. Games) of several monoesters of MM 13902 monosodium salt. The molecular ion was never observed, but in all cases the major ion corresponded to fragmentation (a) (Scheme); the analogous process is well documented in the mass spectra of other β-lactams.[4] Two other fragmentations, (b) and (c), confirmed the presence of the sulphate residue.

Structure elucidation of the second metabolite, MM 4550, was hindered by the difficulty in obtaining a precise empirical formula because of the hygroscopic nature of the compound and its derivatives, although it was evident that the disodium salt contained nitrogen, sulphur, and sodium in the ratio 2:2:2. However, the following spectroscopic data, obtained on the disodio-derivative, permit assignment of structure (**8**); $[\alpha]_D^{20}$ –137° (*c* 0·52 in H_2O); ν_{max} (KBr) 1765 (β-lactam CO), 1695 (amide CO), 1620 (CO_2^-), and 1220—1260br (OSO_3^-) cm^{-1}; λ_{max} (H_2O) 287 (ϵ 12,110) and 240 (13,560) nm. The ^{1}H n.m.r. spectrum in D_2O (rel. to internal standard MeCN, δ 2·00) again revealed the presence of part-structure (**6**) [δ 4·97 {H_a, J(Me-H_a) 6·5, J(H_a-H_b) 9·0 Hz}, 3·88 {H_b, J(H_b-H_c) 6·0 Hz}, 4·37 {H_c, J(H_c-H_d) 9·0, J(H_c-H_e) 10·5 Hz}, 3·46 {H_d, J(H_d-H_e) 18·5 Hz}, 2·99 (H_e), and 1·45 (Me)], and the deshielded methyl group at δ 2·05; the ^{1}H n.m.r. spectrum in $(CD_3)_2SO$ showed the acylenamine system [δ 6·24 (1H, d, J 14 Hz, =CH), 7·18 (1H, dd, J 14 and 11 Hz, NHC*H*=), and 10·65 (1H, br d, J 11 Hz, NH)]. Thus this metabolite was also shown to contain fourteen protons, most of which appeared with chemical shifts and coupling constants virtually identical to those in (**4**); the only significant difference was the position of the *trans*-disubstituted olefinic protons. The ^{13}C n.m.r. spectrum contained thirteen carbon atoms (Table), eight of which showed close correspondence with (**4**). The major differences were seen in the chemical shifts of the four sp^2 carbons, corresponding to the two double bonds, and the methylene carbon.

TABLE. ^{13}C N.m.r. spectra in D_2O.[a]

Carbon	Compound (**4**) δ/p.p.m.	Compound (**9**) δ/p.p.m.
C=O	178 (s)	177 (s)
C=O	172 (s)	173 (s)
C=O	169 (s)	166 (s)
2-C=	144 (s)	140 (s)
NH–CH=	131 (d)	135 (d)
3-C=	128 (s)	139 (s)
S-CH=	103 (d)	112 (d)
>CH–O	74 (d)	74 (d)
5(6)-CH	58 (d)	59 (d)
6(5)-CH	54 (d)	55 (d)
4-CH_2	37 (t)	29 (t)
*Me*CO	23 (q)	23 (q)
*Me*CH–	19 (q)	19 (q)

[a] Dioxan internal standard.

It was apparent from the above evidence that the empirical formula§ of MM 4550 disodium salt (**9**) was $C_{13}H_{14}N_2Na_2O_xS_2$ with $x \geqslant 8$, *i.e.* MM 13902 and MM 4550 were either isomers, or were at a different oxidation level. The possibility of diastereoisomerism was contraindicated by the close similarity of part-structure (**6**) in the ^{1}H n.m.r. spectra of both compounds. Geometrical isomerism could only occur with respect to the configuration of the disubstituted double bond; however, the identical (14 Hz) coupling constant in both cases indicated the same (*trans*) orientation of substituents. No other isomeric possibility exists. It was evident, therefore, that MM 4550 was either the sulphoxide (**8**) or sulphone (**10**) of (**3**). The sulphoxide structure (**8**) was indicated by comparison of the c.d. spectra of the disodium salts; (**4**) had c.d. λ_{max} 222 ($\Delta\epsilon$ –12·54), 264 (+0·33), 292 (–0·66), 326 (–1·24), and 339

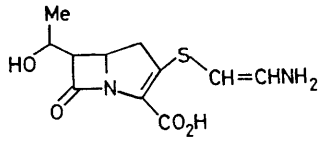
§ Analyses for other commonly encountered elements were negative.

(−0·83) nm, whereas (**9**) had c.d. λ_{max} 210 (−6·24), 234 (+15·60), 261 (−10·92), and 289 (−9·75) nm. This major difference in the c.d. spectra strongly indicated a different number of asymmetric centres, consistent with the presence of the chiral sulphoxide group rather than the achiral sulphone.

The above relationship between (**3**) and (**8**) was finally confirmed by oxidation (*m*-chloroperbenzoic acid–water) of (**4**); the resulting mixture of sulphoxides was shown by spectral and biological comparisons to comprise (**9**) and the diastereoisomeric sulphoxide.

Compounds (**3**) and (**8**) are both powerful inhibitors of a wide range of β-lactamases and also exhibit potent antibacterial properties.

(*Received, 4th May* 1977; *Com.* 431.)



(A)

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