The Structures of the U.V. Chromophoric Fragments of the Antitumour Antibiotics, PD 114,759 and PD 115,028

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Methanolysis of the new and very potent antitumour antibiotics PD 114,759 and PD 115,028 afforded the 3′- and 4′-*N*-(2-methoxypropenoyl)-4,5-dimethoxyanthranilate esters of methyl 2′-deoxy-L-fucopyranoside.

During the course of our antitumour drug discovery programme, a complex of highly potent antitumour antibiotics of novel structure was isolated from the fermentation broth of an *Actinomadura* species (ATCC 39363). As reported earlier[1] the two major components of the complex, PD 114,759 and PD 115,028, are high molecular weight,† sulphur-containing compounds which exhibit excellent activity *in vivo* against P388 lymphocytic leukaemia (%T/C 205 at 0.004 mg/kg) and a variety of solid tumours. Solutions of either PD 114,759 or PD 115,028 in aqueous methanol quickly form an equilibrium mixture containing approximately equal amounts of each antibiotic. The present communication describes our initial efforts concerning the structure elucidation of these antibiotics and reports the structures of the major u.v. chromophoric fragments that are responsible for this interconversion.

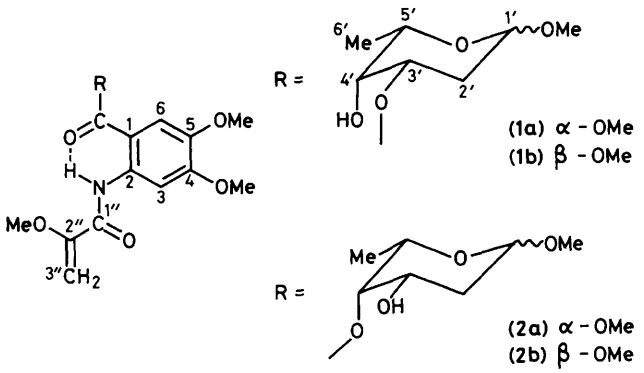
Treatment of PD 114,759 with 0.5 M methanolic HCl at room temperature followed by chromatography over C-18 silica gel gave (**1a**) and (**1b**), both as colourless needles. In a similar manner, PD 115,028 afforded compounds (**2a**) and (**2b**). The u.v. spectra of the products exhibited maxima at 254, 286, and 324 nm, similar to the spectra of the parent antibiotics.[1] The i.r. spectra of these compounds were nearly identical and indicated the presence of a hydroxy (3600 cm^{-1}), an amide NH (3300 cm^{-1}), and two carbonyl groups (1685 and 1680 cm^{-1}). A molecular ion was observed for each compound at *m/z* 425, consistent with the formula $C_{20}H_{27}NO_9$.

The ^1H n.m.r. data for these compounds‡ showed a high degree of similarity. These data, together with information obtained by two dimensional homonuclear and heteronuclear correlation, nuclear Overhauser enhancement, and *J*-resolved experiments, suggested the presence of a methyl 2,6-dideoxyhexopyranoside esterified to an *N*-substituted dimethoxyanthranilic acid moiety. The u.v. maxima and proton chemical shifts of this substituted aromatic system are in close agreement with those for the model compound (**3**) obtained by treatment of 4,5-dimethoxyanthranilic acid with diazomethane followed by acetylation.

Differences in the multiplicities and chemical shifts observed in the ^1H n.m.r. spectra of the methanolysis products were found to be related to carbohydrate protons. In the spectra of (**1a**) and (**2a**), the equatorial H-1′ appears as a broad doublet at δ 4.89 (α-anomers), while a double doublet

† Both PD 114,759 and PD 115,028 exhibit an apparent $M + H$ ion in the fast atom bombardment mass spectrum at *m/z* 1357 using thioglycerol (M_r 108) as a matrix, leading to the original assignment of 1356 as the molecular weight for each. Subsequent studies using a mixture of dithiothreitol and dithioerythritol (M_r 154) as a matrix afforded an apparent $M + H$ ion at *m/z* 1403, indicating that these compounds readily form adducts with the solvent matrix to exhibit ions for ($M + H +$ solvent). The correct molecular weight for PD 114,759 and PD 115,028 is therefore 1248.

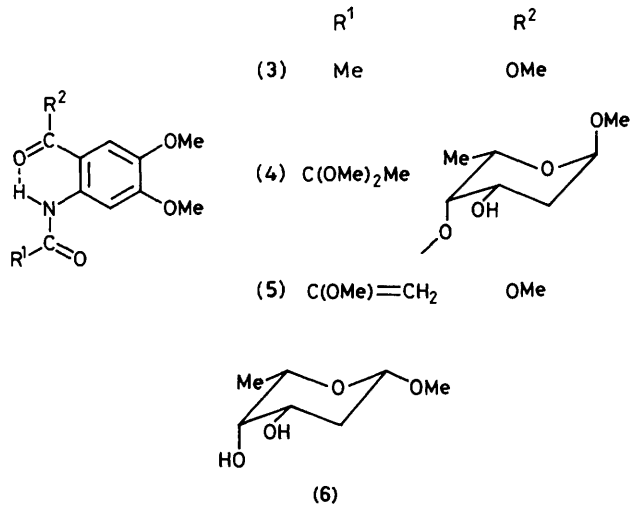
‡ ^1H N.m.r. data ($CDCl_3$, 200 MHz, *J* values in Hz). (**1a**): δ 1.32 (d, *J* 6.5, H-6′), 2.04 (d, *J* 6.4, OH), 2.10 (ddd, *J* 12.4, 5.4, 1.2, H-2′ax), 2.22 (ddd, *J* 12.4, 12.4, 3.5, H-2′eq), 3.38 (s, 1′-OMe), 3.80 (s, 2″-OMe), 3.88 (s, 5-OMe), 3.94 (dd, *J* 6.4, 2.7, H-4′), 3.97 (s, 4-OMe), 4.06 (q, *J* 6.5, H-5′), 4.56 (d, *J* 2.6, H-3″A), 4.89 (br. d, *J* 3.5, 1.2, H-1′), 5.43 (ddd, *J* 12.4, 5.4, 2.7, H-3′), 5.47 (d, *J* 2.6, H-3″B), 7.48 (s, H-6), 8.59 (s, H-3), 11.85 (s, NH); (**2a**): δ 1.96 (m, H-2′), 4.30 (m, H-3′), 4.90 (d, *J* 2.4, H-1′), 5.28 (d, *J* 2.6, H-4′); (**2b**): δ 1.76 (ddd, *J* 12.2, 12.2, 9.6, H-2′ax), 2.09 (ddd, *J* 12.2, 4.8, 2.0, H-2′eq), 3.52 (s, 1′-OMe), 3.72 (dq, *J* 6.5, 0.8, H-5′), 4.03 (m, *J* 12.2, 5.7, 4.8, 3.3, H-3′), 4.42 (dd, *J* 9.6, 2.0, H-1′), 5.24 (d, *J* 3.3, 0.8, H-4′).



at δ 4.42 is observed for the axial H-1′ in compound (**2b**) (β-anomer). These data suggested that C-1′ was the point of attachment to the remainder of the PD 114,759 and PD 115,028 molecules and that displacement by methanol had occurred to yield a mixture of α- and β-anomers. This postulate was confirmed by methanolysis of PD 115,028 in deuteriated solvents. The ^{1}H n.m.r. spectra of the deuteriated products were identical to those of (**2a**) and (**2b**) in all respects except for the absence of the C-1′ methoxy signal. A corresponding increase in the molecular weight (*m/z* 428.1873) was also observed.

The remaining structural difference between (**1a**) and (**2a**) [or (**1b**) and (**2b**)] was readily elucidated by examination of the chemical shifts and coupling constants for H-3′ and H-4′. These data revealed that the dimethoxyanthranilate moiety was attached to C-3′ in (**1a**) and (**1b**) and to C-4′ in (**2a**) and (**2b**). Transformation of (**1a**) or (**2a**) to an equilibrium mixture was readily achieved in aqueous alcoholic solutions, similar to the interconversion of PD 114,759 and PD 115,028. This equilibration was prevented by acetylation, confirming the involvement of the free hydroxy group. It therefore appears likely that PD 114,759 and PD 115,028 are positional isomers capable of interconverting *via* the migration of the anthranilate moiety.

Treatment of (**2a**) with 2.5 M methanolic HCl at room temperature adds methanol to the terminal double bond to give the dimethyl acetal (**4**) (*m/z* 457; $C_{21}H_{31}NO_{10}$). The ^{1}H n.m.r. spectrum of (**4**) exhibited new signals for two aliphatic methoxy groups (δ 3.32, s; 3.33, s) and a methyl group (1.22, s), replacing those assigned to the geminal vinyl protons and the olefinic methoxy in the spectrum of (**2a**). Final confirmation of the proposed structures was obtained by treatment of (**2b**) with 0.5 M methanolic NaOMe, yielding compounds (**5**)



and (**6**). The u.v., i.r., n.m.r., and mass spectra of (**5**) were consistent with the proposed structure, which was confirmed by its synthesis from 2-methoxyacrylic acid[2] and the methyl ester of 4,5-dimethoxyanthranilic acid using 1,1′-carbonyldiimidazole. The isolated carbohydrate (**6**) was found to be identical to an authentic sample of methyl 2-deoxy-β-L-fucopyranoside.

To our knowledge the *N*-substituted dimethoxyanthranilate moiety proposed as a partial structure for PD 114,759 and PD 115,028 is unique among microbial products. Although antibiotics of the pyrrolo[1,4]benzodiazepine class such as tomaymycin and neothramycin[3] contain an anthranilate moiety with the same oxygenation pattern, they are quite unrelated.

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