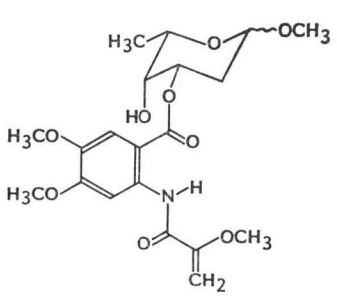
A NOVEL SULFUR-CONTAINING HEXOSE FROM THE ANTITUMOR ANTIBIOTICS, PD 114,759 AND PD 115,028

Sir:

Recently we reported the isolation of PD 114,759 and PD 115,028[1], and the structure of the chromophoric portions of these extremely potent antitumor antibiotics[2]. Further structure studies have revealed that in addition to compounds **1** and **2** (the α and β-anomers of each) obtained by mild methanolysis of PD 114,759 and PD 115,028, respectively, another product, designated factor B4, could be obtained from either antibiotic. Factor B4 is a basic compound and has a molecular weight of 855 (M+H observed at *m/z* 856.3210 by FAB mass spectral analysis), corresponding to a molecular formula of $C_{39}H_{57}N_3O_{12}S_3$ ($C_{39}H_{58}N_3O_{12}S_3$ requires 856.3183). The proposed molecular formula for factor B4 is in excellent agreement with the number of signals observed in its ^1H and ^{13}C NMR spectra.

The structure of factor B4, a moiety present in both PD 114,759 and PD 115,028, could be partially elucidated by examination of several products obtained by further methanolysis. Treatment of factor B4 with methanolic HCl under a variety of conditions, followed by neutralization, afforded several components which were purified by normal or reverse phase chromatography. Two of these products were quite unstable and were tentatively identified as the α and β-methyl glycosides of a 4-(alkylthio)-2,4-dideoxy-3-*O*-methylpentopyranose: ^1H NMR (500 MHz (of the α-anomer), $CDCl_3$) δ 0.97 (3H, d, J=6.4 Hz), 1.05 (3H, d, J=6.2 Hz), 1.47 (1H, ddd, J=3.7, 10.8, 12.8 Hz, 2-H_{ax}), 2.33 (1H, ddd, J=2.0, 4.48, 12.8 Hz, 2-H_{eq}), 2.70 (1H, ddd, J=4.8, 9.7, 9.9 Hz, 4-H), 2.73 (1H, m, J=6.2, 6.4 Hz, possible 4-SC*H*$(CH_3)_2$), 3.35 (3H, s, 3-OCH_3), 3.50 (1H, ddd, J=4.5, 9.9, 10.8 Hz, 3-H), 3.55 (1H, obscured, J=9.7, 10.9 Hz, 5-H_{ax}), 3.75 (1H, obscured, J=4.8, 10.9 Hz, 5-H_{eq}), 5.73 (1H, br dd, J=2.0, 3.7 Hz, 1-H). The ^1H NMR signals of these compounds closely correspond with those observed for an isolated fragment in the 2D ^1H NMR spectrum of factor B4. Although the relative stereochemistry of the pentose could be assigned from the J values measured at 500 MHz, neither of its anomeric methyl glycosides was isolated in sufficient quantity to confirm its structure.

Two additional products were isolated (M^+ = 192 for each) which were identified as the α and β-methyl glycosides (**3** and **4**) of 2,4,6-trideoxy-4-(methylthio)-*lyxo*-hexopyranose. Examination of the decoupled 200 MHz ^1H NMR spectra of $CDCl_3$ solutions of these glycosides led to the following assignments: δ 1.38 (3H, d, J=6.3 Hz, 6-H), 1.89 (dt, J=3.5, 3.6, 14.4 Hz, 2-H_{ax}), 2.13 (ddd, J=1.2, 3.0, 14.4 Hz, 2-H_{eq}),



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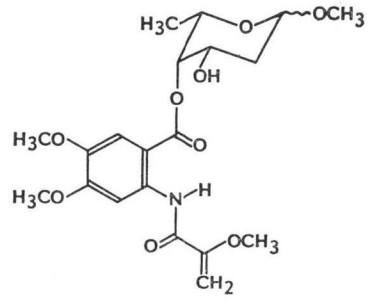

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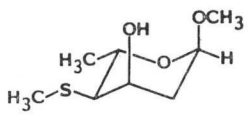

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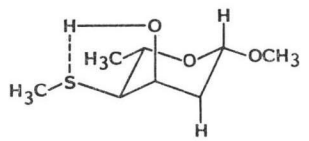

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2.16 (3H, s, 4-SCH_3), 2.37 (dd, J=2.5, 10.7 Hz, 4-H), 3.36 (3H, s, 1-OCH_3), 3.48 (d, J=8.7 Hz, 3-OH), 4.01 (dq, partly obscured, J=6.3, 10.7 Hz, 5-H), 4.06 (m, J=2.5, 3.0, 3.6, 8.7 Hz, 3-H), 4.80 (dd, J=1.2, 3.5 Hz, 1-H) for the α-glycoside (**3**) and δ 1.37 (3H, d, J=6.2 Hz, 6-H), 1.64 (dddd, J=2.3, 3.0, 9.8, 13.6 Hz, 2-H_{ax}), 2.10 (3H, s, 4-SCH_3), 2.24 (ddd, J=2.1, 2.5, 13.6 Hz, 2-H_{eq}), 2.49 (dd, J=2.6, 10.5 Hz, 4-H), 2.75 (dd, J=1.1, 2.3 Hz, 3-OH), 3.48 (3H, s, 1-OCH_3), 3.71 (dq, J=6.2, 10.5 Hz, 5-H), 4.10 (br dd, J=1.1, 2.5, 2.6, 3.0 Hz, 3-H), 4.72 (dd, J=2.1, 9.8 Hz, 1-H) for the crystalline β-glycoside (**4**). The L-configuration is assumed but requires confirmation when more of these anomers become available. The relative stereochemistry was established by a critical analysis of ^{1}H NMR coupling constants and chemical shifts which clearly demonstrated a *cis* relationship for the axial hydroxyl and the equatorial -SCH_3 groups. This configuration explains the unusual W-coupling observed between the 3-OH and the 2-H_{ax} protons in the β-anomer (**4**). This coupling could only occur if the hydroxyl proton is H-bonded to the sulfur at C-4. The observed W-coupling (J= 2.3 Hz) disappeared upon irradiation of the 2-H_{ax} proton or the 3-OH proton in single frequency decoupling experiments. This W-coupling phenomenon is not observed in the α-anomer (**3**), most likely due to a distortion in the chair conformation caused by the axial 1-OCH_3.

The signals corresponding to the 4-methylthiohexose (**4**) and the 4-alkylthio-3-*O*-methylpentose described above are observed in the two dimensional ^{1}H[^{1}H] and ^{13}C[^{1}H] spectra of factor B4. In addition a third sugar, a 4,6-dideoxy-4-mercaptohexopyranose: 2D ^{1}H NMR (500 MHz) δ 1.31 (3H, d, J=6.2 Hz, 6-H), 2.26 (1H, ddd, J=2.1, 9.8, 9.8 Hz (collapses to a dd after exchange with D_2O), 4-H), 3.62 (1H, dd, J=9.8, 9.8 Hz, 2-H), 3.72 (1H, dq, J=6.2, 9.8 Hz, 5-H), 4.02 (1H, br dd, J=9.8, 9.8 Hz (becomes a sharp dd after exchange with D_2O), 3-H), 4.60 (1H, d, J=7.8 Hz, 1-H), 6.88 (1H, d, J=2.1 Hz, 4-SH), was identified from these spectra. Therefore, all three of the sulfur atoms in factor B4 appear to be present as constituents of novel thiosugars. The presence of three sugars is in accord with the ^{13}C NMR spectrum of factor B4 which, in addition to two quaternary carbon signals at 156.0 and 193.8 ppm and six sp^2 carbon signals (three of which are quaternary), exhibits three acetal carbon atom signals at 98.3, 100.7 and 100.8 ppm.

Other sulfur-containing antibiotics with pronounced cytotoxic properties are the epipolydithiodioxopiperazines (gliotoxins and sporidesmins)[3] and the quinomycin/triostin antibiotics[4]. Discounting thioglycosides such as celesticetin and the lincomycins, this is the first report of the presence of thiosugars in antitumor antibiotics and their role in imparting such remarkable activity in the intact PD 114,759 and PD 115,028 antibiotics is of great interest and importance. Additional structural information concerning factor B4 will be reported separately.

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