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TOLYPOMYCIN. II. STRUCTURES OF TOLYPOSAMINE AND TOLYPOMYCIN Y

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Mild acid hydrolysis of tolypomycin Y (I), $C_{43}H_{54}N_2O_{14}$, yielded a water-soluble aminosugar, tolyposamine (III), $C_6H_{13}NO_2$, and a quinone, tolypomycinone (II), $C_{37}H_{43}NO_{13}$ (1). III gave N-benzoyltolyposamine, mp 100-113°, which afforded anomeric methyl N-benzoyltolyposaminides, (XVIIa) and (XVIIb), $C_{14}H_{19}NO_3$, by treatment with 1% of concentrated hydrochloric acid in methanol. They were also obtained by glycosidation of III followed by benzoylation and were indistinguishable from each other in the UV spectra, elemental analysis, mol. peaks in the mass spectra (M^+ m/e 249), and number of the proton signals in the NMR spectra (19), but differed considerably in the following physical properties; i.e., XVIIa was obtained as colorless prisms, mp 182-183°, $[\alpha]_D^{24}$ +10.8 (c=0.5, EtOH), and XVIIb as colorless plates, mp 136-140°, $[\alpha]_D^{24}$ -139° (c=0.5, EtOH). The IR spectra of XVIIa and XVIIb demonstrated the presence of NH (3300 cm^{-1}), CONH (1640), aromatic ring (1535, 700) and -C-O (1080), but the absence of OH, which were similar to each other but not identical.

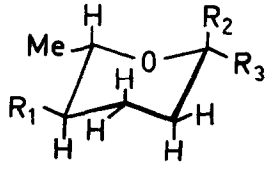
The permanganate oxidation of III yielded ammonia, acetic acid and succinic acid, whereas the periodate oxidation gave acetaldehyde. These data suggested that XVIIa and XVIIb would probably be cyclic sugar derivatives and have one each of $CH-CH_3$, ϕ-CONH, -O-, $C-CH_2-CH_2-C$ and OCH_3 groups in their molecules. The NMR spin-decoupling of XVIIa and XVIIb provided further information as depicted by Fig. 1. The chemical shift, spin-decoupling studies (Fig. 1) and molecular formula of them demonstrated that the methine protons at 4.37 ppm (1H, q)[*1] and 4.68 ppm (1H) should be assigned to the anomeric protons

*1 s = singlet, d = doublet, t = triplet, q = quartet.

of methyl pyranosides. The structure including the carbon skeletons from C_1 to C_4-NHCO·ϕ and the -CH-Me group were corroborated by the NMR spin-decoupling of XVIIa and XVIIb. When the methylene protons in XVIIb were irradiated after addition of D_2O, the methine proton signal at C_4 (3.95 ppm) collapsed to give a doublet (J=9 cps). Similarly, when the methyl protons were irradiated, the methine proton signal at 3.70 ppm collapsed to give a doublet (J=9 cps). These data showed that C_4 should be attached to the -CH-Me group. Thus XVIIa and XVIIb were assigned anomeric methyl 4-benzoylamido-2,3,4,6-tetradeoxyhexopyranosides, respectively.

On a reasonable assumption that both XVIIa and XVIIb have the chair conformation and the fact that the NMR spectra exhibit the $J_{4,5}$ value of 9 cps, it was concluded that the H_4 and H_5 protons lie in a diaxial relationship. The J values between H_1 and H_2 in XVIIa ($J_{1,2\ ax.}$ = 8 cps, $J_{1,2\ eq.}$ = 4 cps) and XVIIb ($J_{1,2\ ax.}$, $J_{1,2\ eq.}$ < 3 cps) showed that H_1 in the former has an axial conformation and H_1 in the latter an equatorial conformation. Furthermore, Hudson's rule (2) discloses that XVIIa and XVIIb should have the β-L-erythro and α-L-erythro structures, respectively. Thus the structure, methyl 4-benzoylamido-2,3,4,6-tetradeoxy-β-L-erythrohexopyranoside, is assigned to XVIIa. Celmer has reported (3) that $J_{1,2}$ of any 6-deoxysugar of α-L-form (1C-type) lies between 1 and 4 cps and the molecular rotation between -263° and -389°. This is in good agreement with our data on XVIIb (α-L-form, $J_{1,2}$ < 3 cps, $[M]_D$ = -346°).

From these facts III is assumed to be 4-amino-2,3,4,6-tetradeoxyhexopyranose.



	R_1	R_2	R_3
(XVIIa)	ϕ·CO·NH-	H-	MeO-
(XVIIb)	ϕ·CO·NH-	MeO-	H-
(III)	H_2N-	H-, HO-	OH-, H- (α+β)

In consideration of tolypomycin Y (I), I should be constructed with tolypomycinone (II) and tolyposamine (III). I, $C_{43}H_{54}N_2O_{14}$, $[\alpha]_D^{21}$ +326° (c=1, EtOH), UV λ_{max}^{EtOH} mμ (ε): 230 (28,700), 290 (23,500), 337 (12,600), IR ($CHCl_3$, cm^{-1}): 3565, 3490 (OH), 3350 (NH), 1725 (-O-CO), 1708, 1680 (CO), 1630 (CO, NHCO),

NMR ($CDCl_3$, ppm): 0.13, 0.63, 1.13 (each 3H, d, CH-Me), 1.84 (3H, s, -C-Me), 1.98 (3H, s, OAc), 2.24 (3H, d, CH=C-Me), 2.30 (3H, s, ϕ-Me), 3.09 (3H, s, OMe), 8.43 (1H, NH), 13.1 (1H, s, chelated OH).

Catalytic reduction of I, a quinoid form, afforded 1,4-dihydrotolypomycin Y (II, a hydroquinoid form, tolypomycin R) and 1,4,16,17-tetrahydrotolypomycin Y (XVIII), a hydroquinoid form, $C_{43}H_{58}N_2O_{14}$. XVIII is oxidized with ferric chloride to give 16,17-dihydrotolypomycin Y (XIX), a quinoid form. The presence of four hydroxyls in I was proved by the stepwise acetylation. All the functional groups in II were observed in I by the IR and NMR spectra, but the UV spectra of II differed from that of I.

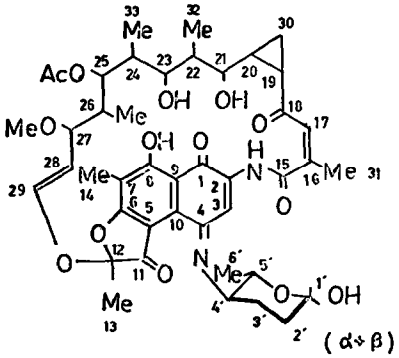
The presence of a carbonyl group on C_{18} was suggested by the observed difference of the UV spectra between I and XIX, and also by the chemical shift of the olefinic protons at C_{17}. Mild alkaline hydrolysis of 1',8-diacetate of I afforded 1'-monoacetate (XX), UV spectrum of which was similar to that of I. All of these acetates of I were readily reduced to afford 1,4-dihydro derivatives, thus excluding the participation of the 1'-hydroxyl group of I to any chemical bonding. The site of the bonding was, therefore, assumed to be C_1, C_4 or C_{11} in II and C_4' or C_5' in III. The anomeric proton was seen as two signals at 4.89 ppm (½H) and 5.30 ppm (½H) respectively, in the NMR spectrum of I. They were decoupled to give two sharp singlets by irradiation of the methylene protons (H_2') at 1.85 ppm. This shows that I is a mixture of two C_1'-anomers*2. When the 6'-methyl protons were irradiated at 1.33 ppm, a methine proton (H_5') at 4.08 ppm collapsed as a doublet, J=9 cps. Therefore, two methine protons at C_4' and C_5' of I should be diaxial, suggesting 1-C conformation.

I was converted by treatment with 1% of concentrated hydrochloric acid in methanol to methyl tolypocyclonide (XXI), $C_{45}H_{58}N_2O_{14}$, UV λ_{max}^{EtOH} mμ (ε): 248 (19,500), 290 (19,200), 330 (sh). Each proton of the sugar part of XXI was similar to that of methyl tolyposaminides. Mild acid hydrolysis of XXI with

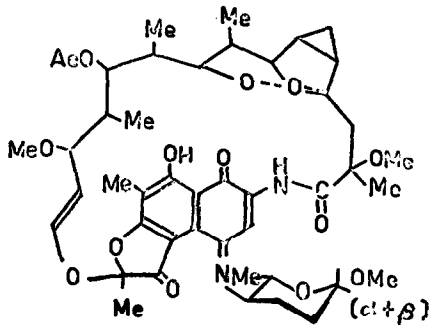
*2 The anomeric proton at 4.89 ppm has an axial conformation and the one at 5.30 ppm has an equatorial conformation judging from their coupling constants. The anomeric proton at 5.73 ppm of XX was ascertained as one proton.

1 mole of hydrochloric acid in 70% methanol gave methoxy tolypocyclonone, (XV). Ammonolysis of XXI gave methyl tolyposaminides, which were derived to two methyl N-benzoyl tolyposaminides, XVIIa and XVIIb. These observations together with the reduction-oxidation of XXI and the acetates of I clearly demonstrated that the site of the bonding between II and III was at the C_4' position.

A hydrogen bonding between the carbonyl group on C_1 and the hydroxyl group at C_8 in I was suggested by the color reaction with magnesium acetate, as well as the IR spectrum (1630 cm^{-1}) and NMR spectrum (13.1 ppm). The C_3-proton in II (7.8 ppm) showed a considerable down-field shift (8.5 ppm) in I. These led us to a conclusion that the structure (I) has to be proposed for tolypomycin Y.



(I)



(XXI)

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