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NEOSIDOMYCIN, A NEW ANTIBIOTIC OF *STREPTOMYCES*

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Summary——A new indole-N-glycoside, neosidomycin (I), is produced by a strain belonging to *Streptomyces hygroscopicus*. The structure of I has been deduced from physico-chemical data obtained using the natural compound, its derivatives and products of degradation reactions.

During the course of our screening program for antibiotics, a new antibiotic, named neosidomycin (I), was isolated from the fermentation broth of a strain belonging to *Streptomyces hygroscopicus*. The antibiotic was weakly active against Gram-negative bacteria, but was not active against Gram-positive bacteria, yeast and fungi. This paper concerns with the structure of neosidomycin (I).

The butanol extract of the culture filtrate (150 liters) was purified by repeated column chromatographies on silica gel and followed Sephadex LH-20 to give I as colorless amorphous powder (yield 2.5 g), $C_{17}H_{20}N_2O_6 \cdot 1/2H_2O$[1]; mp 93-103°C; $[\alpha]_D^{26}$ +51° (c 0.48, MeOH); UV λ_{max} (MeOH) 270 nm (logε 3.91), 279 (3.89), 298 (3.76) and λ_{sh} 283 (3.87); IR (KBr) $\nu_{OH,NH}$ 3360-3450 cm^{-1}, $\nu_{C=O}$ 1745 and 1670 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 7.05-7.70(4H, m), 7.35(1H, s), 6.17(1H, d, J= 9Hz), 4.47(1H, dd, J= 2 and 6Hz), 3.85-4.15(2H, m), 3.70(3H, s), 3.46(2H, s), 2.25(2H, m) and four deuterium exchangeable protons [6.80(2H, m), 5.00(1H, d, J= 4Hz) and 4.98(1H, d, J= 6Hz)].

Treatment of I with 28% aqueous ammonium hydroixde (25°C, 10 min) yielded a diamide (II), colorless needles, $C_{16}H_{19}N_3O_5$; mp 214-216°C; IR (KBr) $\nu_{OH,NH}$ 3200-3400 cm^{-1}, $\nu_{C=O}$ 1655 cm^{-1}. In the IR spectrum, II lacked ester bands (1745 cm^{-1}). The ^{1}H-NMR spectrum of II showed a closed similarity to that of I, except for a lack of a methoxy singlet at δ 3.70 of I. These facts support the presence of a methoxycarbonyl group in I.

Acetylation of I with acetic anhydride/pyridine gave a diacetate (III), $C_{21}H_{24}N_2O_8$; mp 78-80°C; IR (KBr) ν_{NH} 3200-3470 cm^{-1}, $\nu_{C=O}$ 1755, 1745 and 1690 cm^{-1}. The mass spectral fragmentation of III is depicted in Chart. ^{1}H-NMR ($CDCl_3$) δ 7.14-7.75(4H, m), 7.19(1H, s),

5.6-5.8(2H, m, exchangeable with deuterium, 3.86(3H, s), 3.69(2H, s), 2.11(3H, s), 1.67(3H, s) and other six protons as shown in Table 1; ^{13}C-NMR (Table 2).

These spectral data suggest that I is composed of an indole-3-acetamide moiety and a deoxysugar moiety having a methoxycarbonyl group.

The presence of the indole moiety was confirmed as follows. On hydrolysis with 1N hydrochloric acid (100°C, 1 hr), I gave indole-3-acetic acid and ammonium chloride. These two compounds were identified by direct comparisons (IR, TLC, and/or ^{1}H-NMR, mixed fusion).

Although the deoxysugar could not be isolated in pure form from the hydrolysate, first order analysis of the ^{1}H-NMR spectrum of III in comparison with that of I was most informative. As shown in Table 1, all protons of the sugar moiety were assigned in analogy to the ^{1}H-NMR spectral analyses of methyl (methyl-4-deoxy-β-L-*arabino*-hexopyranoside)uronate[2] and its related compounds[3] by aid of spin-decoupling experiments. For instance, the doublet at δ 6.50 in III is attributed to an anomeric proton (1-H) whose coupling constant (9Hz) indicates 1-H and 2-H to be in axial-axial orientation. These assignments reveal that the acetylated sugar moiety has, in $CDCl_3$ solution, a *C1* conformation[2,3] with β-glycosidic linkage and that the sugar is methyl 4-deoxy-D(or L)-*ribo*-hexopyranuronate[4] which was found as a natural product for the first time.

The remaining problem is the position of the glycosidic linkage in the indole moiety. In the ^{13}C-NMR spectrum of III, the anomeric carbon was observed at δ 76.7 showing a N-C-O glycosidic linkage[5]. The ^{1}H-NMR spectrum of III lacked NH proton of indole ring, whereas two deuterium exchangeable amide protons were found at δ 5.6-5.8. Furthermore, the IR spectra of I, II and III exhibited the broad primary amide bands at about 1655-1690 cm^{-1} and lacked amide II bands at 1500-1600 cm^{-1} being characteristic for secondary amides. These results indicate that the deoxysugar moiety is linked to N-atom of the indole ring. In addition, the fact that one acetyl signal of III resonanced at higher field (δ 1.67) was in accordance with a molecular model study in which C-2 acetyl group located above the indole ring having an anisotropic effect.

Consequently, the structure of I was deduced to be methyl [1-(3-carbamoylmethylindoyl)-4-deoxy-D(or L)-*ribo*-hexopyranoside]uronate, except for its absolute configuration.

The N-glycosidic linkage of I resembles to that of nucleosides, but the occurence of indole-N-glycoside as a compound of biological origin has not been reported hitherto.

Chart

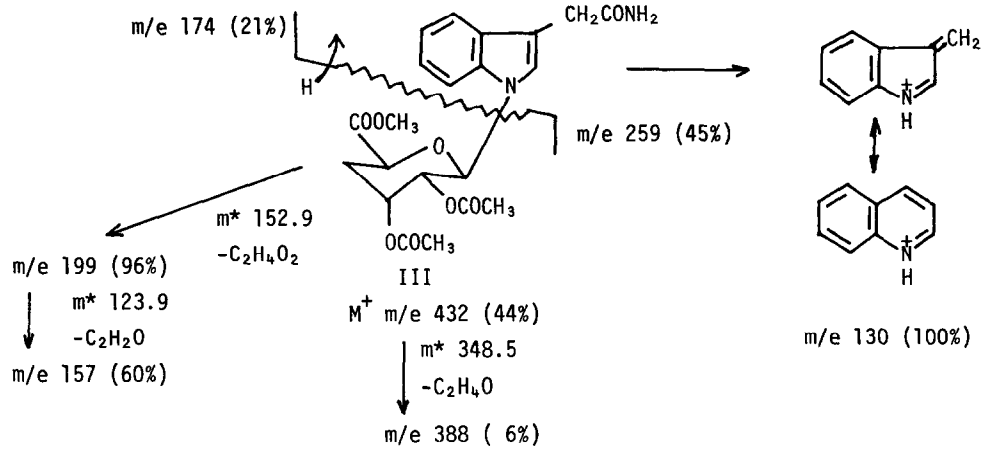
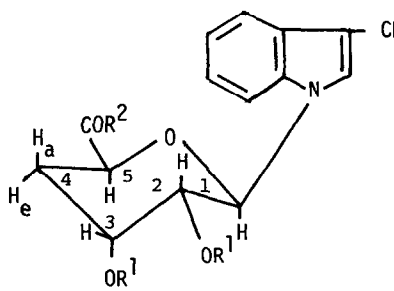


Table 1. Chemical Shift and Coupling Constant Values of Diacetate (III) ($CDCl_3$)

Protons		H-1	H-2	H-3	$H\text{-}4_a$	$H\text{-}4_e$	H-5
Coupling Constant	(Hz)		9.5	3.1	2.9	15.0	2.1
					3.7		
					6.6		
Chemical Shift	(δ)	6.50	5.37	5.54	2.38	2.67	4.61
Splitting		d	dd	dq	dq	dq	dd
Spin Decoupling		irr → d					
				irr → dd		dd	
				irr → d		d ← irr	
				d ← irr	irr → s		

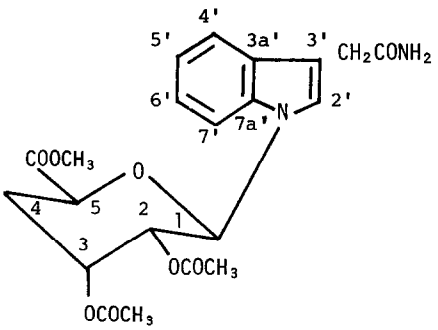
Abbreviations; ↑—↑=coupling, s=singlet, d=doublet, dd=double-doublet, dq=double-quartet, irr=irradiated proton(s).



	R^1	R^2
I	H	OCH_3
II	H	NH_2
III	$COCH_3$	OCH_3

Table 2. ^{13}C-NMR Data of Diacetate (III) ($CDCl_3$)

Chemical Shifts (δ)	Splitting Pattern[a)]	Assigment
174.1[b)]	s	$\underline{C}OOCH_3$
171.5[b)]	s	$\underline{C}ONH_2$
169.5	s	} $O\underline{C}OCH_3$ x2
169.4	s	
137.3	s	C-7'a
128.0	s	C-3'a
123.3[c)]	d	C-2'
123.1[c)]	d	C-5'
120.9[d)]	d	C-4'
119.1[d)]	d	C-6'
111.0	s	C-3'
110.3	d	C-7'
76.7	d	C-1
70.3[e)]	d	C-5
68.8[e)]	d	C-2
67.9[e)]	d	C-3
52.3	q	$COOC\underline{H}_3$
33.0[f)]	t	C-4
31.0[f)]	t	$\underline{C}H_2CONH_2$
20.7	q	} $\underline{C}H_3CO$ x2
20.3	q	



a) On off-resonance proton decoupling. s=singlet, d=doublet, t=triplet, q=quartet.
b)~ f) Assignments for these peak positions may be reversed.

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References and Notes

1). Satisfactory elemental analyses were obtained for all new compounds.

2). H. W. H. Schmidt and H. Neukom, *Tetrahedron Letters*, 1964, 2063.

3). Idem, *Carbohyd. Res.*, 10, 361, (1969).

4). The corresponding alcohol, 4-deoxy-D-*ribo*-hexose, has been synthesized. M. Černý, J. Staněk and J. Pacák, *Coll. Czech. Chem. Comm.*, 34, 1750, (1969).

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