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Shigeharu Inouye*1 : Optical Rotatory Dispersion Curves of N-Salicylidene Derivatives of Amino-sugar Antibiotics.*2

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The optical rotatory dispersion (ORD) curves of N-salicylidene-amino-sugars in the monosaccharide series were reported in the accompanying paper[1]. This paper will show how the ORD curves of N-salicylidene derivatives readily derivable from the amino-sugar antibiotics can be used as a means of identification of oligosaccharide amino-sugars.

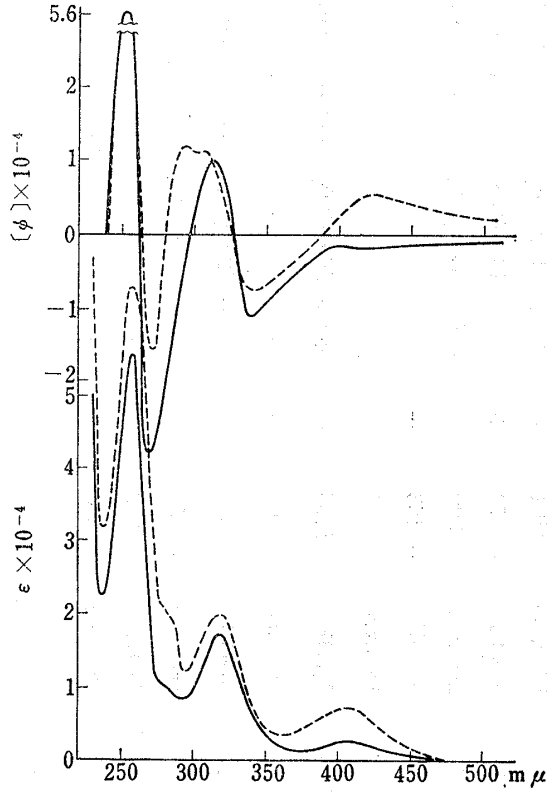


Fig. 1. ORD Curves and Electronic Spectra of N-Salicylidene-kanamycin (I) (——) and -kanamycin B (II) (- - -) in Methanol

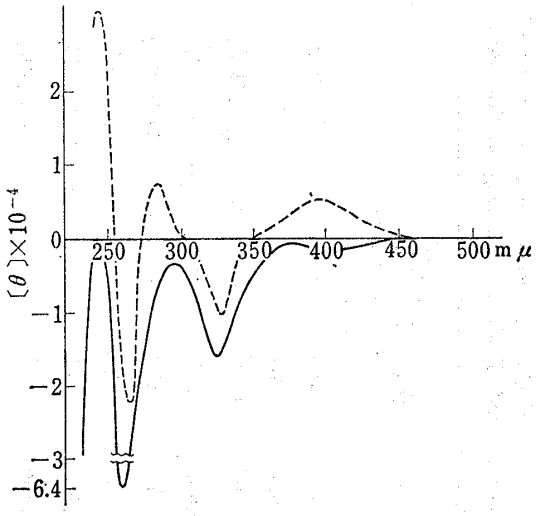


Fig. 2. CD Curves of N-Salicylidene-kanamycin (I) (——) and -kanamycin B (II) (- - -) in Methanol

Table I summarized molecular rotations at the sodium D-line ($[\phi]_D$), signs of Cotton effects and molecular ellipticities ($[\theta]$) at the maximum circular dichroism (CD) curve in N-salicylidene derivatives examined in this paper, together with $[\phi]_D$ of the parent compounds. Fig. 1 showed the ORD curves and electronic absorption spectra of tetra-N-salicylidene-kanamycin (I) and penta-N-salicylidene-kanamycin B (II) in methanol. The Schiff base I showed negative plain curve in the visible region and three negative Cotton effects associated with the 406, 318 and 257 mμ bands in the electronic spectrum.

*1 Morooka, Kohoku-ku, Yokohama-shi (井上重治).

*2 A part of this work was presented at the 9th Symposium on the Chemistry of Natural Products held at Osaka on October 13, 1965. "Symposium Abstracts," p. 7.

1) S. Inouye : This Bulletin, **15**, 1557 (1967).

TABLE I. Molecular Rotations ($[\phi]_D$), Signs of Cotton Effects and Molecular Ellipticities ($[\theta]_{max}$) in N-Salicylidene Derivatives of Amino-sugar Antibiotics and Their Partial Degradation Products in Methanol and Dioxane

N-Salicylidene derivative of	$[\phi]_D$ of parent amino-sugar (H_2O)	Solvent	$[\phi]_D$	Sign of plain ORD 600~450 mμ	Sign of Cotton effect ($[\theta]_{max}$ (mμ))			
Kanamycin (I)	+620°	MeOH	− 160°	−	−(− 1700(402))	−(−16000(322))		−(−63000(258))
		Dioxane	− 261	−		−		−
Kanamycin B (II)	+610	MeOH[a)]	+1130	+	+(+ 5100(400))	−(−10000(326))	+(+ 7300(285))	−(−22000(265))
		Dioxane	+ 690	+	+(+ 800(408))	−(−23000(323))	+	−
Neomycin B (III)	+436	MeOH	− 459	−	−(−12000(408))	−(−14000(317))		−(−23000(256))
		Dioxane	+ 805	+	+(+ 200(405))	−(−16000(317))	+(+18000(265))	−(−29000(252))
Neomycin C (IV)	+675	MeOH	+ 731	+	−(− 2000(408))	−(− 6900(323))		−(−21000(257))
		Dioxane	+ 644	+	+(+ 530(408))	−(−16000(324))		
Paromomycin-I (V)	+380	MeOH	− 636	−	−(−11000(408))	−(−11000(319))		−(−20000(256))
		Dioxane	+1191	+	+(+ 150(408))	−(− 5200(322))		−(−24000(265))
Paromomycin-II (VI)	+610	MeOH	+ 579	+	−(− 1300(408))	−(−16000(324))		−
		Dioxane	+ 990	+	+(+ 600(410))	−(− 1500(317))	+(+ 3000(304))	+(+20000(252))
O-α-D-3-Amino-3-deoxyglucopyranosyl-(1→6)-2-deoxystreptamine (VII)	+310	MeOH	− 153	−	−			
		Dioxane	− 64	−		−		−
O-α-D-2,6-Diamino-2,6-dideoxyglucopyranosyl-(1→4)-2-deoxystreptamine(VIII)	+365	MeOH[a)]	+1005	+	+(+ 2600(395))	−(− 6100(322))	+(+ 8600(276))	
		Dioxane	+ 590	+	+	−	+	−
Methyl 3-O-(α-L-2,6-Diamino-2,6-dideoxyidopyranosyl)-β-D-ribopyranoside (IX)	− 27	MeOH	− 570	−	−(− 5200(408))	−(− 6000(318))		−(−14000(257))
		Dioxane	+ 160	+	+(+ 24(410))	−(− 6000(318))	+(+ 3000(274))	−(− 9000(256))
O-α-D-2-Amino-2-deoxyglucopyranosyl-(1→4)-2-deoxystreptamine (X)	+369	MeOH[a)]	+1632	+	+	+	+	+
		Dioxane	+1386	+	+	+		+

a) Contained 20% dimethylformamide.

The assignment of negative sign for the weak Cotton effect near 406 mμ was not definite in the ORD curve, but, obvious in the CD curve shown in Fig. 2. The ORD curve of I in dioxane solution was almost identical with that in methanol, except for the Cotton effect near 406 mμ, which disappeared in parallel with the intensity decrease of the 406 mμ band in the electronic spectrum.

N-Salicylidene Schiff base of kanamycin B (II) exhibited, both in methanol and in dioxane, positive dispersion in the transparent region and four Cotton effects in the 257~406 mμ region, corresponding to the four absorption bands in the electronic spectrum [257, 280 (shoulder), 318 and 406 mμ].*3 The relatively strong Cotton effect near 406 mμ in methanol was probably associated with the strong absorption of the 406 mμ band. The striking difference in the ORD curves of I and II in the visible region, which arised from the opposite signs in the Cotton effects near 406 mμ (Fig. 2), was significant, since both of the ORD curves of the parent antibiotics in water exhibited plain dispersion with close $[\phi]$ values, as shown in Fig. 3.

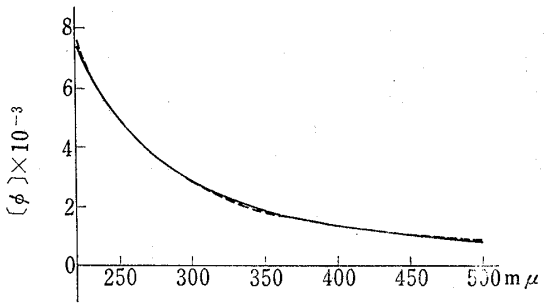


Fig. 3. ORD Curves of Kanamycin (——) and Kanamycin B (- - -) in Water

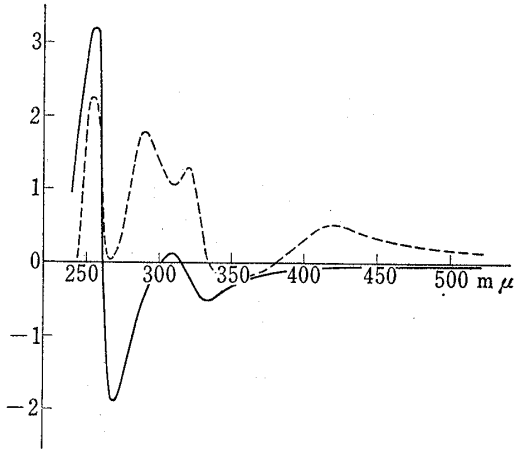
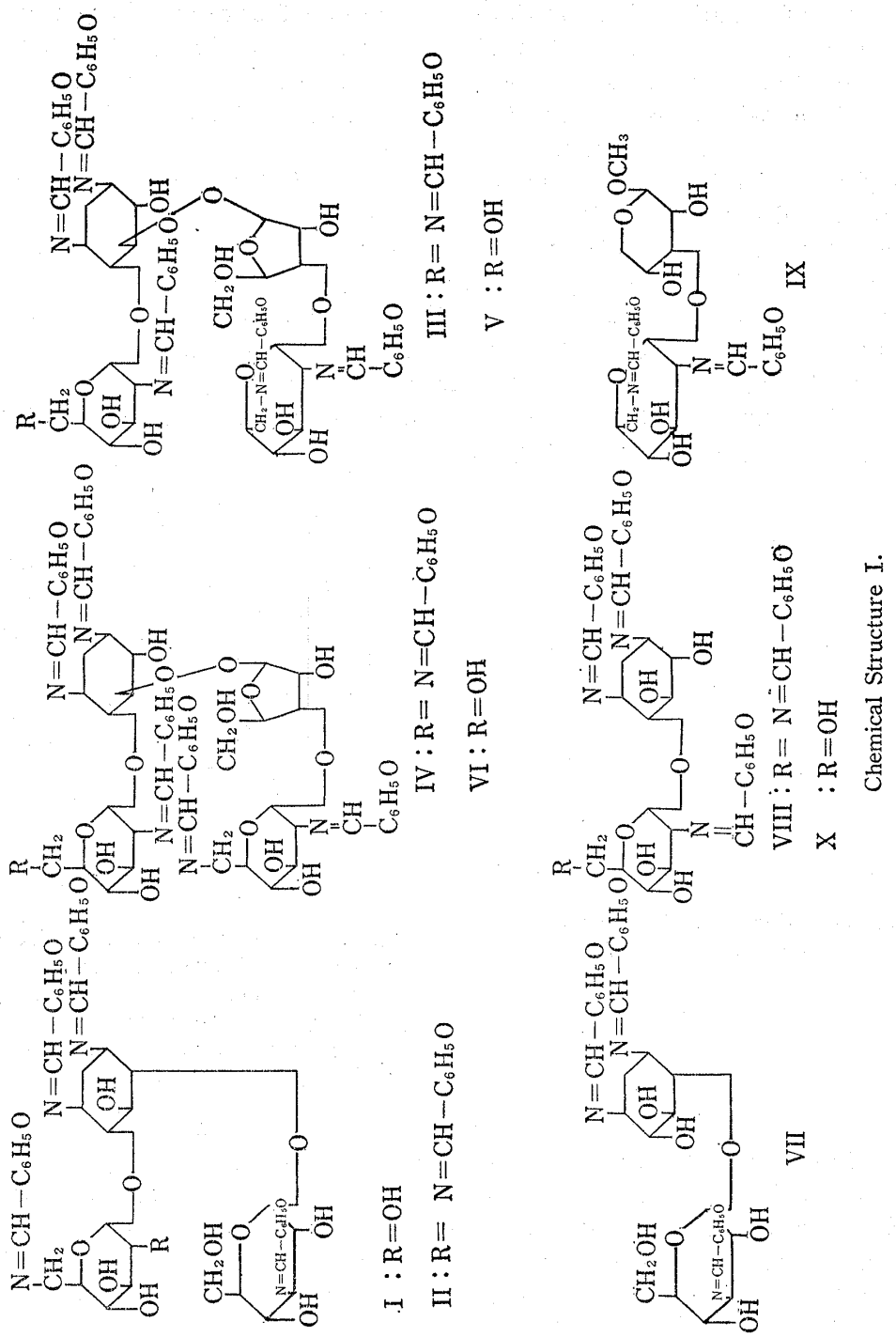


Fig. 4. ORD Curves of N-Salicylidene-O-α-D-3-amino-3-deoxyglucopyranosyl-(1→6)-2-deoxystreptamine (VII) (——) in Dioxane and N-Salicylidene-O-α-D-2,6-diamino-2,6-dideoxy glucopyranosyl- (1→4)-2-deoxystreptamine (VIII) (- - -) in Methanol

In order to examine the rotational contribution of the component chromophores in I and II, we have measured the ORD curves of the Schiff bases of partial degradation products of the antibiotics. Fig. 4 showed the ORD curves of N-salicylidene derivatives of O-α-D-3-amino-3-deoxyglucopyranosyl-(1→6)-2-deoxystreptamine (VII), a common component of I and II, and O-α-D-2,6-diamino-2,6-dideoxyglucopyranosyl-(1→4)-2-deoxystreptamine (VIII), a component of II. The Schiff base VII exhibited very similar ORD curve to that of I, showing negative dispersion in the visible region followed by the three negative Cotton effects in methanol and two negative Cotton effects in dioxane. Since the Cotton effect caused by the 6-salicylideneamino-α-D-glucopyranose moiety in I was expected to be positive in sign,[1] its rotational contribution would be small in I.

The ORD curve of VIII, which was obtained by the removal of the 3-salicylideneamino-glucose moiety from II, displayed positive Cotton effects centered around 400 and 280 mμ and negative Cotton effects around 320 and 255 mμ, similar to the ORD curve of II. In this case, therefore, the contribution of the 3-salicylideneaminoglucose moiety that should give rise to negative Cotton near 280 and 400 mμ seemed to be relatively small.

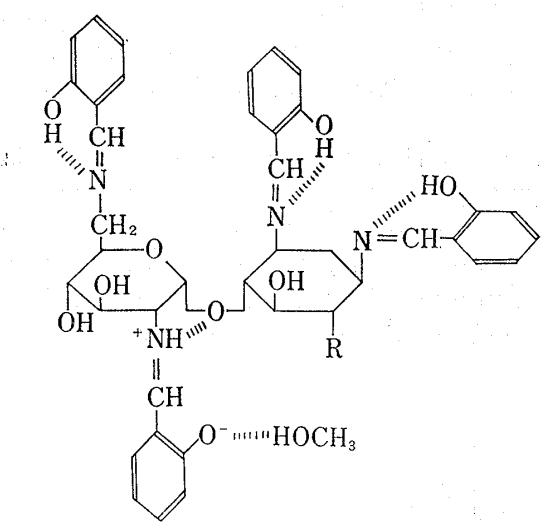
*3 The CD curve of II in methanol disclosed an additional positive maximum near 243 mμ, which apparently corresponded to no absorption band. The probable origin of the extra maxima including also the positive maximum near 265 mμ in III and near 304 mμ in VI in dioxane was discussed in reference 1.



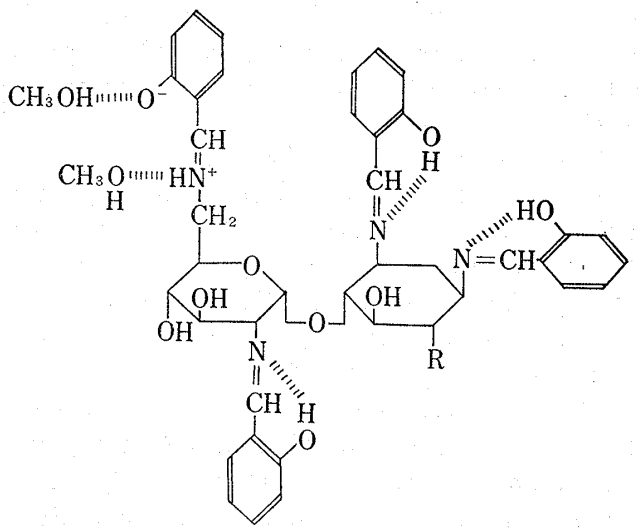
Chemical Structure I.

The alternative inversion of the signs of the four Cotton effects in II and VIII indicated the opposite contribution of the optically active ketoamine and phenolimine chromophores equilibrated in solution, because the electronic transitions at 280 and 405 mμ were due to the π-π^* of the ketoamine species and the transitions at 255 and 315 mμ to the π-π^* of the phenolimine species.[2] A calculation of the relative amounts of the two tautomers in VIII (and II) using the similar procedure as did for the di-N-salicylidene-amino-sugars suggested that the most predominant ketoamine species in methanol were a and b, in which the C-2 or C-6 chromophore in the diamino-glucose moiety took the ketoamine and the remaining chromophores took the phenolimine (Fig. 5). The most dominant

2) S. Inouye: This Bulletin, **15**, 1540 (1967).



Ⅷa : R=OH
Ⅱa : R=3-Salicylideneamino-α-D-glucose(phenolimine form)



Ⅷb : R=OH
Ⅱb : R=3-Salicylideneamino-α-D-glucose(phenolimine form)

Fig 5. Schematic Representation of Structures of the Predominant Ketoamine Species of Ⅷ and Ⅱ in Methanol

phenolimine species, on the other hand, was c in which all the chromophores in the molecule took the phenolimine.*4 The positive Cotton effects near 405 and 280 mμ, therefore, may be determined, as a first approximation, by the rotatory contribution of the two ketoamine chromophores in the diamino-sugar, whereas, the negative Cotton effects near 255 and 315 mμ may be the composite result influenced by all the optically active phenolimine chromophores in the molecule.

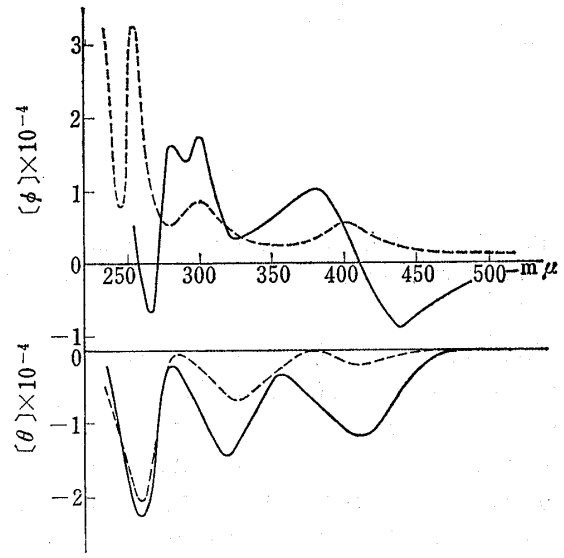


Fig. 6. ORD and CD Curves of N-Salicylidene-neomycin B (Ⅲ) (——) and -neomycin C (Ⅳ) (- - -) in Methanol

While the parent neomycins B and C showed similar positive dispersions in water, their N-salicylidene derivatives (Ⅲ and Ⅳ) exhibited large difference in the ORD curves, particularly in the visible region in methanol (Fig. 6). Thus, Ⅲ showed negative $[\phi]_D$ and negative dispersion in the transparent region, followed by three negative Cotton effects. The Schiff base Ⅳ, on the contrary, showed positive $[\phi]_D$ and positive dispersion, followed by three negative Cotton effects. As clearly demonstrated in the CD curves in Fig. 6, the ORD difference between Ⅲ and Ⅳ arised from the difference in the rotational strength of the negative Cotton effects at the longest wave-length band. The negative dispersion with negative $[\phi]_D$ in Ⅲ was undoubtedly due to the large amplitude of the negative Cotton effect near 405 mμ, while the positive dispersion with positive $[\phi]_D$ in Ⅳ was not

*4 Approximate percentages of the main tautomers of Ⅷ in methanol, calculated assuming the microscopic equilibrium constants of 0.09 for the two chromophores in the deoxystreptamine moiety, 0.31 for the C-6 chromophore and 0.38 for the C-2 chromophore in the 2,6-diaminohexose portion, were 18% for a, 14% for b and 47% for c. It could be generally shown that, when the macroscopic equilibrium constant (K_T value in p. 6) was much lower than an unity, the percentages of the tautomers having polyketoamine chromophores became negligible.

the direct reflection of the weakly negative Cotton effect, but, of the positive background rotation of the parent antibiotic. The large CD maximun at 400 mμ in Ⅲ, as compared to Ⅳ, seemed to be related, at least partly, to the strong absorption band at 405 mμ in the electronic spectrum.

The ORD difference observed in the neomycin group (Ⅲ and Ⅳ) was seen as such in the salicylidene derivatives of paromomycin-I (Ⅴ) and paromomycin-II (Ⅵ) in methanol. Again, Ⅴ which contained the 2,6-disalicylideneamino-α-L-idopyranose moiety as a common component to Ⅲ, exhibited negatively rotatory dispersion with negative $[\phi]_D$ and strongly negative CD maximum at 410 mμ, while Ⅵ which contained the 2,6-disalicylideneamino-α-D-glucopyranose moiety common to Ⅳ, showed positive dispersion with positive $[\phi]_D$ and small CD maximum (Table I).

Differed from the kanamycin group, the ORD curves of the neomycin and paromomycin group displayed notable solvent effect. As exemplified in Fig. 6 and 7, the negative dispersion with negative $[\phi]_D$ in Ⅲ (and Ⅴ) in methanol changed to the positive dispersion with positive $[\phi]_D$ when measured in dioxane solution. The solvent effect for the ORD curve of Ⅳ (and Ⅵ) was rather small, showing positive dispersion in the visible region both in methanol and in dioxane. However, the CD curve shown in Fig. 7 revealed the solvent-induced inversion of the sign near 410 mμ in Ⅳ as well as Ⅲ, though the sign near 320 mμ remained in negative. An analogous solvent effect was observed in the case of methyl N-salicylidene-3-O-(α-L-2,6-diamino-2,6-dideoxy-idopyranosyl)-β-D-ribopyranoside (Ⅸ), a methanolysis product of Ⅲ and Ⅴ. The ORD curve of Ⅸ exhibited negative dispersion in the visible region with negative $[\phi]_D$ value in methanol and positive dispersion with positive $[\phi]_D$ in dioxane, similar to Ⅲ and Ⅴ. The CD curve illustrated in Fig. 8 indicated that the negative CD maximum associated with the 405 mμ band in methanol became positive in dioxane, while the negative CD maximum near 320 mμ remained little unchanged in two solvents. Thus, the rotatory contributions of the ketoamine and phenolimine species of Ⅸ as well as Ⅲ～Ⅵ were opposite in dioxane.

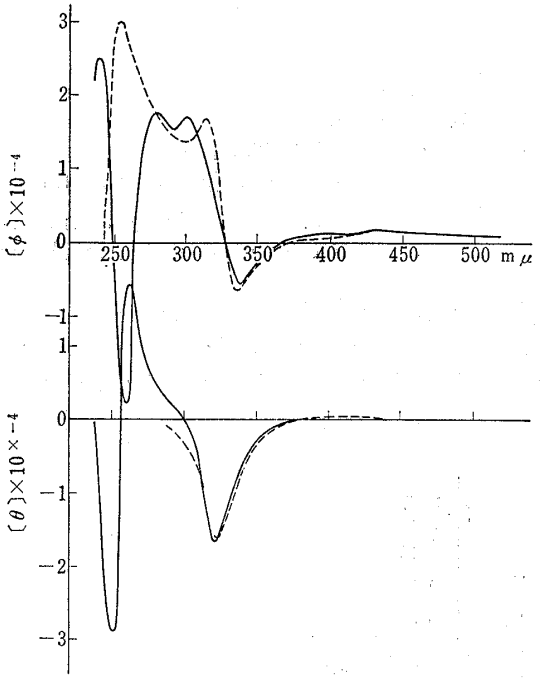


Fig. 7. ORD and CD Curves of N-Salicylidene-neomycin B (Ⅲ)(——) and -neomycin C (Ⅳ)(···) in Dioxane

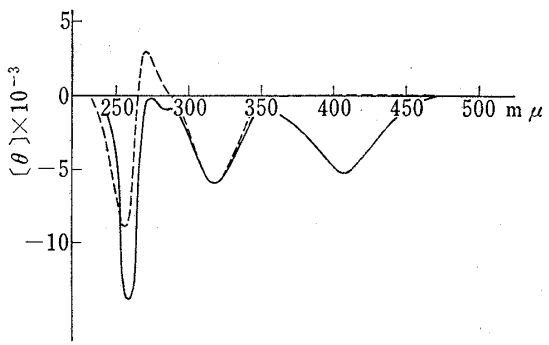


Fig. 8. CD Curves of Methyl N-Salicylidene-3-O-(α-L-2,6-diamino-2,6-dideoxyidopyranosyl)-β-D-ribopyranoside (Ⅸ) in Methanol (——) and in Dioxane (- - -)

These results suggested that the N-salicylidene chromophores in the 2,6-diamino-α-L-idose moiety linked to D-ribose provided the largest contribution to the ORD and CD of Ⅲ and Ⅴ. Indeed, the optical rotatory contribution of the component Ⅷ to the ORD of Ⅲ would be small, since it should give rise to positive Cotton near 410 mμ in methanol. The common component to Ⅴ and Ⅵ, that is, N-salicylidene-O-α-D-2-amino-2-deoxyglucopyranosyl-(1→4)-2-deoxystreptamine (Ⅹ), exhibited positive $[\phi]_D$ and three positive Cotton

TABLE II. Optical Rotatory Dispersion Data of N-Salicylidene Derivatives of Amino-sugar Antibiotics and Their Partial Degradation Products in Methanol and Dioxane

Compound	Solvent	$[\phi]_{589}$	$[\phi]_{450}$	$[\phi]$ (mμ) at peak and trough in ORD curve								
I	MeOH	− 210	−1300	− 1800 (420)	− 1600 (397)	−11000 (339)	+10000 (313)	−30000 (268)	+56000 (252)			
I	Dioxane	− 300	−1100	− 2000 (410)	− 2000 (400)	−12000 (339)	+11000 (310)	−30000 (268)	+56000 (254)			
II	MeOH	+1400	+4600	+ 5500 (420)	− 7800 (343)	+11000 (308)	+11000 (303)	+12000 (293)	−16000 (272)	+56000 (252)		
II	Dioxane	+ 600	+1100	+ 1200 (440)	−10000 (336)	+17000 (310)	+14000 (285)	−59000 (261)	+48000 (230)			
III	MeOH	− 420	−7000	− 8600 (438)	+10000 (382)	+ 3200 (335)	+17000 (300)	+14000 (291)	+16000 (280)	− 6800 (266)		
III	Dioxane	+ 780	+1600	+ 1700 (432)	+ 1400 (409)	+ 1400 (404)	− 5600 (340)	+17000 (300)	+16000 (293)	+17000 (275)	−28000 (259)	+25000 (239)
IV	MeOH	+ 850	+1600	+ 5600 (400)	+ 2600 (358)	+ 8400 (300)	+ 5200 (278)	+33000 (255)	+ 7800 (247)			
IV	Dioxane	+ 710	+1700	+ 1900 (434)	+ 500 (386)	− 6200 (336)	+17000 (314)	+14000 (300)	+30000 (255)			
V	MeOH	− 620	−8200	− 9200 (439)	+ 9500 (375)	+ 2300 (340)	+19000 (303)	+11000 (290)	+32000 (249)			
V	Dioxane	+1200	+2800	+ 3700 (400)	+13000 (307)	+10000 (298)	+25000 (270)	−32000 (253)				
VI	MeOH	+ 650	+2000	+ 1400 (436)	+ 3200 (405)	− 1300 (340)	+19000 (310)	+14000 (301)	+24000 (282)			
VI	Dioxane	+1100	+3000	+ 3400 (428)	+ 3200 (405)	+ 4300 (355)	+16000 (320)	+10000 (300)	+32000 (267)	−27000 (246)		
VII	MeOH	− 150	− 700	− 1600 (400)	− 1600 (390)	− 4000 (350)						
VII	Dioxane	− 60	− 400	− 5200 (336)	+ 1300 (309)	−16000 (270)	+32000 (255)					
VIII	MeOH	+1300	+3900	+ 4800 (415)	− 1800 (350)	+13000 (320)	+11000 (312)	+18000 (290)	0 (267)	+22000 (254)		
VIII	Dioxane	+ 630	+1300	+ 1400 (446)	+ 1200 (430)	−11000 (340)	+ 1500 (305)	+13000 (294)	+14000 (278)	−66000 (258)	+12000 (245)	
IX	MeOH	− 560	−3800	− 4900 (432)	+ 4500 (375)	+ 160 (334)	+ 8400 (297)	− 7000 (266)				
IX	Dioxane	+ 200	+ 290	+ 70 (400)	− 4300 (338)	+ 8000 (295)	− 1600 (266)	+12000 (250)				
X	MeOH	+1500	+7000	+10000 (408)	+ 200 (370)	+ 8000 (318)	+ 7000 (306)	+15000 (290)	+11000 (275)	+27000 (262)		
X	Dioxane	+1400	+4300	+ 4800 (432)	+ 4100 (392)	+12000 (324)	+ 7200 (304)	+44000 (265)	0 (250)			

effects in methanol and no dioxane-induced inversion of sign, implying the slight contribution to the ORD curve of V. The tautomeric equilibrium position (K_T) of the compounds I~X calculated from the equation $K_T=0.69\times\frac{A_{405}}{A_{318}}$ [2] were summarized in Table III. The higher K_T value, and hence higher ketoamine content in IX than VII and X suggested the larger contribution of the idose portion to the 405 mμ band of III and V.

It was evident from the above results that the ORD curves of N-salicylidene amino-sugars in the ketoamine form can provide more characteristic Cotton effects than in the phenolimine form, and can be used as a means of identification of amino-sugars.

Experimental

N-Salicylidene derivatives employed in this work were prepared according to the procedure described in the separate paper.[2] Tetra-N-salicylidene-kanamycin (I)[3] crystallized from MeOH had m.p. 260~265° (decomp.). Penta-N-salicylidene-kanamycin B (II) crystallized from MeOH showed m.p. 268~270°. *Anal.* Calcd. for $C_{53}H_{57}O_{15}N_5$: C, 63.4; H, 5.7; N, 7.0. Found: C, 63.2; H, 5.4; N, 6.9. Hexa-N-salicylidene-neomycin B (III) was obtained as an amorphous state from hot EtOH. *Anal.* Calcd. for $C_{65}H_{69}O_{19}N_6$: C, 63.1; H, 5.6; N, 6.8. Found: C, 61.9; H, 6.3; N, 6.8. Hexa-N-salicylidene-neomycin C (IV), crystallized from MeOH-iso-PrOH, m.p. >200° (decomp.). *Anal.* Calcd. for $C_{65}H_{69}O_{19}N_6$: C, 63.1; H, 5.6; N, 6.8. Found: C, 61.9; H, 6.5; N, 6.7. Penta-N-salicylidene-paromomycin-I (V) crystallized from iso-PrOH, m.p. >185° (decomp.). *Anal.* Calcd. for $C_{58}H_{64}O_{19}N_5$: C, 61.4; H, 5.7; N, 6.2. Found: C, 59.4; H, 6.0; N, 6.1. Penta-N-salicylidene-paromomycin-II (VI) was precipitated from EtOH-iso-PrOH. *Anal.* Calcd. for $C_{58}H_{64}O_{19}N_5$: C, 61.4; H, 5.7; N, 6.2. Found: C, 59.7; H, 6.4; N, 6.4. Tri-N-salicylidene-O-α-D-3-amino-3-deoxyglucopyranosyl(1→6)-2-deoxystreptamine (VII), m.p. 163~165°.[4] Tetra-N-salicylidene-O-α-D-2,6-diamino-2,6-dideoxyglucopyranosyl-(1→4)-2-deoxystreptamine (VIII), m.p. 198~201°.[5] Methyl di-N-salicylidene-

Table III. Electronic Absorption Data of N-Salicylidene Derivatives in Methanol and Dioxane

Compound	Solvent	λ_{max} mμ ($\varepsilon\times10^{-4}$)				K_T[a]
I	MeOH	257 (5.56)		318 (1.72)	406 (0.26)	0.11
I	Dioxane	257 (5.32)		318 (1.84)		
II	MeOH	257 (6.47)	280 (sh)	318 (2.00)	406 (0.70)	0.24
II	Dioxane	257 (7.10)		318 (2.37)		
III	MeOH	256 (6.30)	280 (sh)	318 (1.86)	406 (1.08)	0.40
III	Dioxane	257 (7.95)		318 (2.70)		
IV	MeOH	256 (7.33)	280 (sh)	318 (2.25)	405 (0.99)	0.30
IV	Dioxane	257 (7.80)		318 (2.64)		
V	MeOH	257 (5.97)	280 (sh)	318 (1.75)	405 (1.08)	0.42
V	Dioxane	258 (6.55)		319 (2.20)		
VI	MeOH	257 (6.10)	280 (sh)	318 (1.77)	405 (0.75)	0.29
VI	Dioxane	257 (7.00)		319 (2.35)		
VII	MeOH	257 (4.43)		318 (1.42)	404 (0.15)	0.07
VII	Dioxane	257 (4.05)		317 (1.47)		
VIII	MeOH	256 (5.48)	280 (sh)	316 (1.72)	405 (0.64)	0.26
VIII	Dioxane	257 (5.08)		317 (1.80)		
IX	MeOH	256 (2.84)	280 (sh)	317 (0.85)	407 (0.47)	0.38
IX	Dioxane	257 (2.88)		318 (0.92)		
X	MeOH	257 (4.17)	280 (sh)	317 (1.26)	405 (0.47)	0.26
X	Dioxane	257 (4.23)		318 (1.41)		

a) K_T=(Ketoamine)/(Phenolimine).

3) M. J. Cron, D. L. Johnson, F. M. Palermiti, Y. Perron, H. D. Taylor, D. F. Whitehead, I. R. Hooper: J. Am. Chem. Soc., **80**, 752 (1958).
4) S. Inouye: This Bulletin, in press.
5) T. Ito, M. Nishio, H. Ogawa: J. Antibiotics, Ser. A, **17**, 189 (1964).

3-O-(α-L-2,6-diamino-2,6-dideoxyidopyranosyl)-β-D-ribopyranoside (IX) was re-precipitated from hot iso-PrOH. *Anal.* Calcd. for $C_{26}H_{32}O_{10}N_2$: C, 58.6; H, 6.1; N, 5.3. Found: C, 57.9; H, 6.1; N, 5.0. Tri-N-salicylidene-O-α-D-2-amino-2-deoxyglucopyranosyl-(1→4)-2-deoxystreptamine (X) was crystallized from MeOH. m.p. 178~180°. *Anal.* Calcd. for $C_{33}H_{37}O_{10}N_3$: C, 62.3; H, 5.9; N, 6.6. Found: C, 61.3; H, 6.6; N, 6.4.

The ORD and CD measurements were performed on a JASCO Model ORD/UV-5 instrument using quartz cells of 1.0 cm thickness at 20~25°. Unless otherwise stated, the concentrations used were 0.4~0.2% in the 600~450,mμ region, 0.2~0.04% in the 450~350 mμ region, 0.01~0.008% in the 350~280 mμ region and 0.003~0.002% in the 280~230 mμ region. ORD Data were summarized in Table II and the electronic absorption data recorded on a Hitachi EPS-2U spectrometer were listed in Table III

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