OXAMICETIN, A NEW ANTIBIOTIC OF BACTERIAL ORIGIN

II. STRUCTURE OF OXAMICETIN

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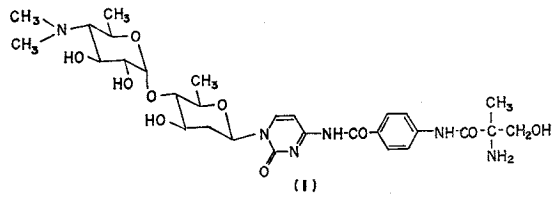
The structure of oxamicetin, $C_{29}H_{42}N_6O_{10}$, has been determined including the streochemistry. It is closely related to amicetin with 3-hydroxyamicetose (D-chromose C or olivose) in place of amicetose in amicetin.

Oxamicetin is a new antibiotic elaborated by *Arthrobacter oxamicetus* sp. nov. Studies on the production and isolation of the antibiotic and a comparison with amicetin have been reported[1]. This paper reports the structure of oxamicetin (**I**).



(I)

General Structural Characteristics

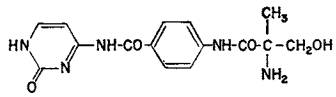
Oxamicetin (**I**) is a basic antibiotic which crystallizes as a hydrochloride hydrate. Elemental analysis and titration data[1] were consistent with a molecular formula of $C_{29}H_{42}N_6O_{10}$ for the free base, indicating one additional oxygen atom compared to amicetin. Oxamicetin shows strong absorption in the ultraviolet region with, spectra at various pH values[1] very similar to those of amicetin[2]. The IR spectrum[1] showed absorptions due to hydroxyl groups (3300 cm^{-1}), amide carbonyls (1690, 1640) and conjugated C=C bonds (1600 cm^{-1}). Oxamicetin gives positive ninhydrin, DRAGENDORFF and anthrone reactions but is negative to FEHLING'S and TOLLENS' reagents, indicating the absence of reactive reducing sugar moieties. The NMR spectrum of oxamicetin hydrochloride in deuterium oxide showed three C-CH_3 (two doublets centered at δ 1.44 and 1.55 ppm, one singlet at δ 1.78 ppm) and one dimethylamino group at δ 3.09 ppm[1]. The characteristic two vinyl protons appeared as doublets at δ 7.32 (J=7.5 Hz) and 8.09 ppm (J=7.5 Hz) and four aromatic protons as an AB quartet at δ 7.58 and 7.80 ppm (J=9.0 Hz). In addition, two anomeric protons were observed at δ 5.32 (doublet, J=3.5 Hz) and 5.70 ppm (broad doublet, J=10.5 Hz). These NMR data were also suggestive of the close structural similarity of this antibiotic to amicetin.

Degradation Studies

Methanolysis—Isolation of cytimidine (**II**), methyl amosaminide (**IV**) and compound **III**

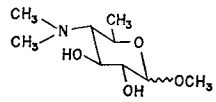
Oxamicetin (**I**) was hydrolyzed in methanolic hydrogen chloride under conditions similar to

those used for amicetin[3] (room temperature for 40 hours). The resulting precipitate was converted to the free base (**II**), which was identified as cytimidine by UV, IR and NMR spectra and by mixture melting point determination with an authentic sample.



II *Cytimidine*

The filtrate from the above methanolyzate was evaporated *in vacuo* and the concentrate was adsorbed on a column of Dowex 50W×4 (H^+). The column was eluted with methanol followed by methanolic ammonia and the fractions containing sugar fragments were detected by anthrone reagent. The anthrone-positive fractions were concentrated to an oil, which was treated with chloroform to separate it into an insoluble solid (**III**) and and a soluble oil (**IV**). The latter was fractionally crystallized from a mixture of ether and petroleum ether to give a basic sugar (colorless needles, **IVa**), which melted at 89~93°C and was identical with methyl α-amosaminide by IR spectrum and mixture melting point determination with an authentic sample. From the mother liquor, an anomer (**IVb**) was obtained as the crystalline hydroloride (m.p. 198~205°C, dec.) and identified as methyl β-amosaminide.



IV *Methyl amosaminide*

Structure of compound III

Compound **III** described above was further purified by column chromatography (Dowex 1×2, OH^-) and obtained as a colorless crystalline solid which melted at 137~140°C. It analyzed for $C_{10}H_{15}N_3O_4$, had pKa′ value of 4.15 and showed UV absorption maxima at 278.5 nm in 0.1 N HCl

Table 1. UV and pKa′ data for **III** and related compounds

Compounds	UV λ_{max}(nm)		pKa′
	in acid	in alkali	
Cytosine[4,18]	274	272 (pH 12) 282 (pH>13)	4.45~4.60
1-Substituted cytosine			
Compound **III**	278.5	270	4.15
Compound **V**	278	270	4.0 & 7.2
1-Methyloytosine[4,18]	283	274	4.45
Cytidine[4,18]	280	272.5	4.1
Cytosamine[5]	278	270	3.9 & 7.0
Blastocidin S[16]	275	266~270	
Gougerotin[16]	276	269	
3-Substituted cytosine			
3-Methylcytosine[6,18]	274	294	7.38

Fig. 1. NMR spectrum of III-HCl (60 MHz, in D_2O)

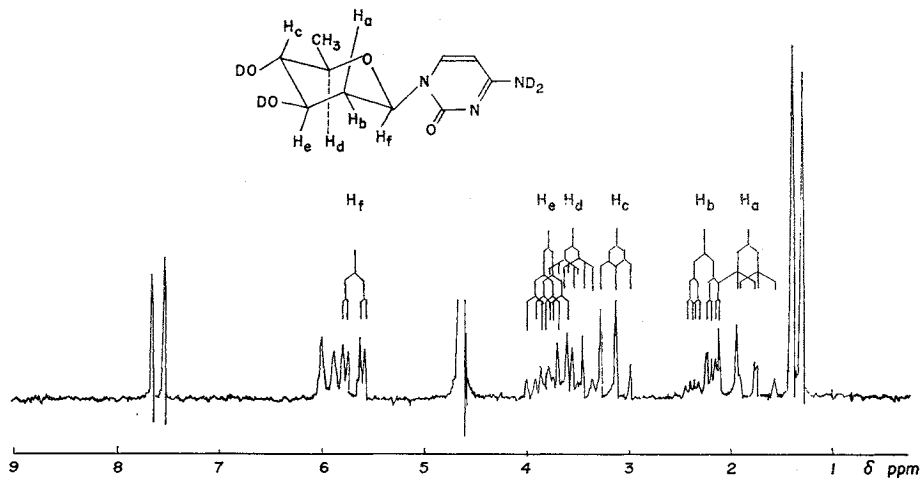
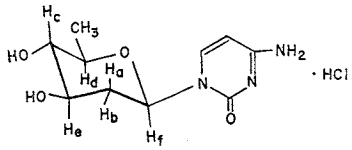
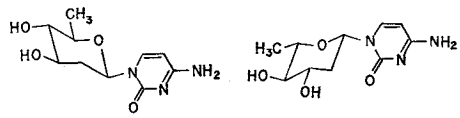


Table 2. NMR parameters of compound **III** (60 MHz, in D_2O)



Assignment	Chemical shift (δ, ppm)	Coupling constant (Hz)
H_a	1.85 (quartet)	J_{ab} =12.5 J_{af} =10.5 J_{ae} =10.5
H_b	1.28 (double quartet)	J_{ba} =12.0 J_{bf} = 2.4 J_{be} = 5.1
H_e	3.15 (triplet)	J_{ce} = 9.0 J_{cd} = 9.0
H_d	3.59 (doublet of quartets)	J_{dc} = 9.0 J_{dCH_3} = 6.2
H_e	3.82	J_{ea} =10.5 J_{ec} = 9.0 J_{eb} = 5.1
H_f	5.75 (doublet of doublets)	J_{fa} =10.5 J_{fb} = 2.4
CH_3	1.35 (doublet)	J_{CH_3d} = 6.2
Cytosine ring protons	6.02, 7.70 (doublet)	J = 8.0

and at 270 nm in 0.1 N NaOH. Compound **III** gave positive reactions with ninhydrin and anthrone reagents but was negative to FEHLING's solution. These properties suggested a sugar-cytosine structure for compound **III**, but the negative FEHLING's reaction along with the acid stability of **III** excluded the possibility of an O-glycosidic linkage[15] in the compound. As shown in Table 1, the UV spectrum and the pKa′ value of **III** are similar to those of 1-methylcytosine[4], cytosamine[5] and other 1-substituted cytosines[18] but quite different from those of 3-methylcytosine[6], suggesting a 1-glycosylcytosine structure for compound **III**. The NMR spectrum of **III** (Fig. 1) was analyzed as shown in Table 2, which led to the following two possible structures, **IIIa** and **IIIb**.



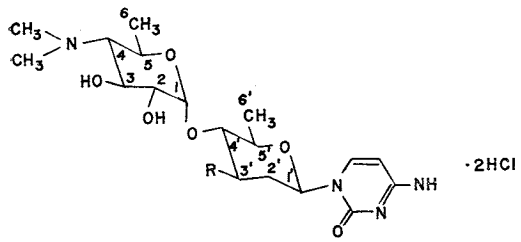
IIIa IIIb

It was further determined by the copper complex method[7] that the configuration **IIIa** was to be assigned to the compound. The rotational shift of compound **III** in aqueous cuprammonium solution (1.6% cuprous chloride in 15 N ammonia), $\Delta[M]_{436}$, was determined to be +2370, and this large positive value clearly indicated that the projected angle between the two hydroxyl groups at C-3 and C-4 is +60°. Thus, the structure of compound **III** is 1-(2, 6-dideoxy-β-D-*arabino*-hexopyranosyl) cytosine, or more simply 1-(β-3-hydroxyamicetosyl) cytosine.

Alkaline hydrolysis of oxamicetin — Isolation of compound **V**

Oxamicetin was hydrolyzed in 0.2 N sodium hydroxide solution at room temperature. The basic fragment in the hydrolyzate was extracted with 1-butanol, purified by ion-exchange chromatography (Dowex 1×2, OH^-), and crystallized from aqueous acetone to give colorless prisms (**V**) which melted at 172~174°C. It analyzed for $C_{18}H_{30}N_4O_7$ and showed a UV absorption maximum at 278 nm in 0.1 N HCl and at 270 nm in 0.1 N NaOH. Compound **V** gave positive ninhydrin and negative FEHLING's reactions. These properties and the IR spectrum of **V** are very similar to those of cytosamine[5,8,9], but cytosamine and **V** were clearly differentiated by TLC and NMR spectra. With alumina TLC developed with 80% methanol, **V** showed R_f 0.39 and cytosamine R_f 0.45. The NMR data of **V** (in D_2O, 60 MHz) are shown in Table 3 compared to those of cytosamine. From the above data, compound **V** was assumed to have the amosamine moiety glycosidically linked to one of the two hydroxyl groups of compound **III**. Furthermore, the magnitude of the coupling constant of the anomeric proton (J=3.5 Hz, doublet) indicated an α-linkage of amosamine.

Table 3. NMR parameters of compound **V** and cytosamine (60 MHz, in D_2O)



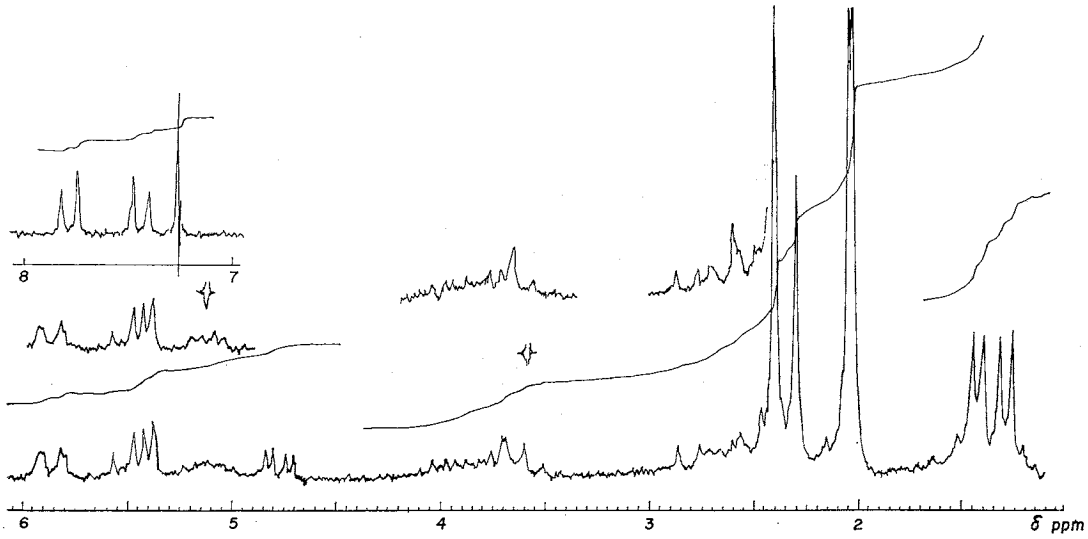
Assignment	Chemical shift (δ: ppm)	
	Compound **V** R = OH	Cytosamine R = H
1′-H	5.67 (1H)	5.66 (1H)
2′-H_2	1.5~2.5 (2H)	1.6~2.6 (4H)
3′-H_2	—	
1-H	5.25 (1H)	5.05 (1H)
4-$N(CH_3)_2$	2.95 (6H)	2.98 (6H)
6 and 6′—CH_3	1.30 (3H)	1.26 (3H)
	1.41 (3H)	1.45 (3H)
Cytosine ring protons	6.16 (1H)	6.18 (1H)
	7.88 (1H)	7.89 (1H)

Tetraacetylhydroxycytosamine (**VI**)

In order to determine which one of the two hydroxyl groups of 3-hydroxyamicetose is linked to amosamine, compound **V** was acetylated with acetic anhydride-pyridine at room temperature[12] to give its tetraacetyl derivative ($C_{26}H_{38}N_4O_{11}$, **VI**), which showed a UV spectrum similar to that of N-acetyl-1-methylcytosine[13], having maxima at 212.5, 249 and 300 nm.

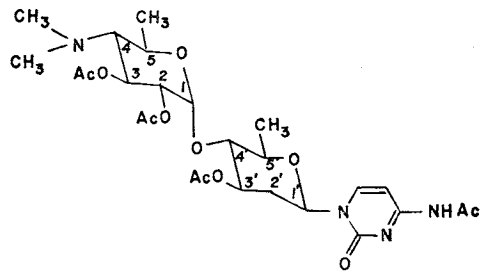
The 100 MHz NMR spectrum of **VI** (Fig. 2) indicated that acetylation resulted in a down-field shift of three protons of **V**. Two of them appeared at δ 4.77 ppm (doublet of doublets, J=10.0 and 4.0 Hz) and at δ 5.46 ppm (triplet, J=10.0 Hz), and were assigned to the C-2 and C-3 hydrogens of

Fig. 2. NMR spectrum of **VI** (100 MHz, in $CDCl_3$)



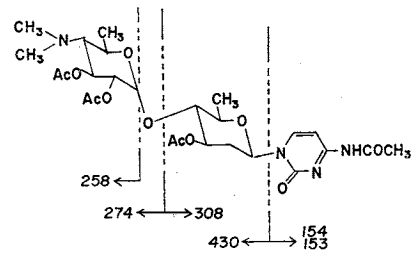
amosamine, respectively. The other proton, which appeared as a multiplet centered at δ 5.14 ppm, was considered to be the C-3′ or C-4′ hydogen of the 3-hydroxyamicetose moiety in compound **VI**.

Irradiation of a proton at δ 3.58 ppm, which was later attributed to the C-4′ hydrogen, caused the multiplet signal at δ 5.14 ppm to collapse to a doublet of doublets (J=10.4 and 4.6 Hz), and the splitting pattern indicated that this quartet proton was still coupled with the vicinal equatorial and axial protons of C-2′. Furthermore, irradiation at δ 5.14 ppm caused changes of the splitting pattern of a multiplet at δ 2.4~2.7 ppm (methylene region) as well as of a triplet at δ 3.58 ppm (C-4′ proton).



VI *Tetraacetyl-hydroxycytosamine*

Thus, the multiplet at δ 5.14 ppm was assigned to the C-3′ hydrogen and, therefore, amosamine must be linked to the C-4′ hydroxyl group of 3-hydroxyamicetose, as in the case of amicetin where amosamine links to the C-4′ hydroxy of amicetose. In the mass spectrum of **VI**, the molecular ion was observed at *m/e* 582 and the other fragment ions were consistent with the proposed structure of **VI** as illustrated below. The fragment ions of the pyrimidine base which appeared at *m/e* 154 (base +2H) and *m/e* 153 (base +1H) are in accord with results from other nucleosides[14]. Accordingly, compound **V** is hydroxycytosamine in accordance with the designation of the corresponding fragment from amicetin.



VI Tetraacetylhydroxycytosamine

Since the structure of cytimidine, which was obtained by methanolysis of oxamicetin, has been established, structure (**I**) is assigned to oxamicetin.

Discussion

The present study has assigned the structure of oxamicetin, which is closely related to that of amicetin. Several *Streptomyces* species have been reported to produce amicetin[16] and two other related antibiotics, bamicetin[17] and plicacetin (amicetin B)[17]. These three *Streptomyces* antibiotics contain the same sugar component amicetose or 2, 3, 6-trideoxy-D-*erythro*-hexose. The corresponding sugar moiety in oxamicetin has been determined to be 2, 6-dideoxy-D-*arabino*-hexose, which was designated 3-hydroxyamicetose. It should be noted that the latter sugar, 2, 6-dideoxy-D-*arabino*-hexose, has been reported as a constituent of chromomycins and olivomycin, and named D-chromose C[10] and olivose[11], respectively.

It is interesting to note that compound **III**, 1-(3-hydroxyamicetosyl)-cytosine, was stable toward acid hydrolysis under conditions which were found to cleave the amicetose-cytosine linkage in amicetin. This is in accord with the fact that the pyrimidine nucleosides of glucose are stable to acid hydrolysis[19], and that a glycosidic bond of 3-deoxyglucose is easily hydrolyzed as compared with glucose[20].

Experimental

Isolation of cytimidine (**II**) A solution of oxamicetin hydrochloride (1.081 g) in 20 ml of methanol was cooled below −10°C in a Dry Ice-acetone bath. The solution was saturated with dry hydrogen chloride gas and then kept at room temperature for 40 hours. Cytimidine hydrochloride, which deposited in the dark purple solution, was collected by filtration, washed with methanol and dried *in vacuo*; yield, 304 mg, mp 262°C (dec.). The hydrochloride (100 mg) was dissolved in 5 ml of water, and the solution was decolorized with activated charcoal and made alkaline (pH 8.2) with aqueous sodium hydroxide solution to afford 52 mg of crystalline cytimidine (II); mp 265.5~267.5°C (dec.), no depression in the mixture melting point determination with cytimidine,* $\lambda_{max}^{0.1N\,HCl}$ 243 nm (ε 10,200) and 320.5 nm (ε 27,000), $\lambda_{max}^{0.1N\,NaOH}$ 271.5 nm (ε 16,200). The analytical sample was recrystallized from hot water.

Methyl amosaminide (**IVa, IVb**) The filtrate of the above methanolyzate was concentrated to 5 ml and applied to a column of Dowex 50W × 4 (H^+, 1.5 × 40 cm) which had been washed with methanol. The column was fractionally eluted with methanol, then with methanolic ammonia (1.5 N) solution. A trace amount of neutral sugar was obtained from the methanolic eluate, but was insufficient for characterization. The anthrone-positive fractions eluted by methanolic ammonia solution were collected and evaporated *in vacuo* to dryness. The residue was treated with 20 ml of chloroform and an insoluble solid (**III**, 105 mg) wa collected by filtration. The chloroform solution was evaporated *in vacuo* to afford 250 mg of a crude anomeric mixture of methyl amosaminides (**IV**).

The crude **IV** (200 mg) was dissolved in 10 ml of ether, treated with active charcoal, and concentrated *in vacuo* to 3 ml. Petroleum ether was added to the solution to the cloud point. After

* The authentic samples of cytimidine was prepared from amicetin by the published method[9].

standing overnight in a refrigerator, the white crystals (**IVa**) which separated were collected by filtration and recrystallized from the same solvent; mp 89~93°C, $[\alpha]_D^{25}$+130° (*c* 0.4, H_2O). A mixture of **IVa** with authentic methyl α-amosaminide* showed no melting point depression.

The mother liquor of **IVa** was collected and concentrated to dryness. The residual oil was converted to its hydrochloride salt by treating with 5 ml of methanolic hydrogen chloride solution (2 N) and evaporating the solvent. The residue was crystallized from a mixture of ethanol and ether to give 10 mg of colorless cubes (**IVb**), mp 198~205°C, no melting point depression with an authentic specimen of methyl β-amosaminide hydrochloride*.

1-(3-Hydroxyamicetosyl)cytosine (**III**) A solution of crude **III** (90 mg) in 3 ml of water was loaded on a column of Dowex 1×2 (OH^-, 0.7 × 35 cm). The column was developed with water and the eluate was collected in 3-ml fractions. The fractions which gave positive anthrone reaction were collected, concentrated and lyophilized to give 31.5 mg of white crystalline powder (**III**); mp 137~140°C. $[\alpha]_D^{25}$ −4° (*c* 0.38, H_3O). $\lambda_{max}^{0.1N\,HCl}$ 278.5 nm (ε 11,900), $\lambda_{max}^{0.1N\,NaOH}$ 270 nm (ε 8,120), IR (KBr) 3400, 1660, 1610, 1500 and 1080 cm^{-1}.

Anal. Calcd. for $C_{10}H_{15}N_3O_4 \cdot 1/2H_2O$: C, 47.99; H, 6.44; N, 16.79.
Found: C, 47.94; H, 6.38; N, 16.42.

Hydroxycytosamine (**V**) A solution of oxamicetin hydrochloride (940 mg) in 20 ml of 0.2 N sodium hydroxide was stirred overnight at room temperature. The reaction mixture was extracted with two 10 ml portions of 1-butanol. The combined extracts were washed with a small amount of water and concentrated *in vacuo* to afford 290 mg of crude solid. The product was charged on a column of Dowex 1×2 (OH^-, 1.0 × 35 cm) and developed with water. The anthrone-positive fractions were combined, concentrated *in vacuo* and lyphilized to give 199 mg of hydroxycytosamine (**V**), which was crystallized from aqueous acetone solution; mp 172~174°C, $\lambda_{max}^{0.1N\,HCl}$ 278 nm (ε 13,500), $\lambda_{max}^{0.1N\,NaOH}$ 270 nm (ε 8,500), IR (KBr) 3400~3300, 1655, 1610, 1495, 1380, 1293 and 1075 cm^{-1}.

Anal. Calcd. for $C_{18}H_{30}N_4O_7$: C, 52.16; H, 7.30; N, 13.52.
Found: C, 51.98; H, 7.79; N, 12.77.

Tetraacetylhydroxycytosamine (**VI**) A solution of **V** (86 mg) in 1 ml of acetic anhydride and three drops of pyridine was heated in an oil bath (110°C) for 6 hours. The reaction mixture was cooled, then poured into ice water (10 g) to decompose the excess acetic anhydride. The solution was adjusted to pH 8.2 with 6 N sodium hydroxide under cooling and white precipitate which deposited was collected by filtration. The precipitate was crystallized from a mixture of ethyl acetate and *n*-hexane to give 58 mg of colorless needles (**VI**); mp 158~159°C; λ_{max}^{EtOH} 212.5 nm (ε 20,200), 249 nm (ε 17,800) and 300 nm (ε 7,600); IR (KBr) 1750, 1675, 1630, 1495, 1370, 1240 and 1040 cm^{-1}.

Anal. Calcd. for $C_{26}H_{38}N_4O_{11}$: C, 53.60; H, 6.57; N, 9.62.
Found: C, 53.87; H, 6.50; N, 9.24.

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* The authentic samples of methyl α- and β-amosaminide, and cytosamine were prepared from amicetin by the published method[3].

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