BICYCLOMYCIN, A NEW ANTIBIOTIC
II. STRUCTURAL ELUCIDATION AND ACYL DERIVATIVES

TAKASHI KAMIYA, SHIZUO MAENO, MASASHI HASHIMOTO and YASUHIRO MINE

Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan

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The structure of bicyclomycin, a novel antibiotic, was largely elucidated by nuclear magnetic resonance spectroscopy and established finally as 8,10-diaza-6-hydroxy-5-methylene-1-(2′-methyl-1′, 2′, 3′-trihydroxy-propyl)-2-oxabicyclo [4, 2, 2] decan-7, 9-dione by X-ray crystallographic diffraction analysis. A number of its acyl derivatives were prepared and studied biologically. The relationship between the structure of the acyl derivatives and the rate of urinary excretion was also examined.

Bicyclomycin, produced by *Stereptomyces sapporonensis* ATCC 21532, is a novel antibiotic with inhibitory activity against Gram-negative bacteria. The isolation and characteristics of this antibiotic were reported in the preceeding paper[1]. The present paper deals with the structural elucidation and biological properties of its derivatives.

Structural Elucidation

The IR spectrum of bicyclomycin shows absorptions at 3400, 3300 and 3160 cm^{-1} due to -NHCO- or -OH stretching vibrations. Amide carbonyl group absorptions are found at 1703 and 1673 cm^{-1}. The NMR spectrum shows a singlet due to $\equiv C\text{-}CH_3$ proton at τ 8.76. The one-proton doublet at τ 6.03 (J=7.5 Hz) and the two-proton broad singlet at τ 6.45 are assigned to protons at hydroxy-bearing carbons. These signals shifted to τ 4.69 and 5.94 respectively on acetylation.

The relationship of the two OH groups and the CH_3 group was provided by oxidative degradation. By HIO_4 oxidation, formaldehyde and acetic acid were obtained in good yield. This result indicates the presence of a 2-methyl-1,2,3-trihydroxypropyl group in the molecule. A hemiacetal was isolated as a third product from the reaction mixture. The structure was provided by the following facts. It gave a crystalline 2,4-dinitrophenylhydrazone, although its IR-spectrum shows no carbonyl absorption except amide carbonyl absorption. On catalytic hydrogenation, this hemiacetal gave a carbinol. Its NMR spectrum shows an AB-type quartet at τ 6.01 and 6.36 (J_{AB}=11.0 Hz) due to $-CH_2OH$ bonded to an asymmetric quaternary carbon.[2] From these results, the presence of the unit $\equiv C\text{-}CH(OH)\text{-}CCH_3(OH)\text{-}CH_2OH$ in the molecule was established.

The NMR spectrum of bicyclomycin shows the presence of terminal olefinic protons at τ 4.89 and 4.60 as broad singlets and two methylenic protons at τ 7.40 and

6.10 as multiplets. These methylenic protons are ascribable to $=\underline{C}-CH_2-$ and $-O-CH_2-$ groups respectively. Catalytic hydrogenation and spin decoupling study gave further information with regards to these groups. Bicyclomycin absorbs one mole of hydrogen on catalytic hydrogenation and gives dihydrobicyclomycin, m.p. 191~192°C, $C_{12}H_{20}O_7N_2$. Its NMR spectrum shows a three-proton signal due to $-\underline{CH}-\underline{CH_3}$ protons as a doublet at τ 8.90 (J=7.0 Hz) and a one-proton multiplet due to $-\underline{CH}-CH_3$ at τ 7.73. The methylenic protons appear as two two-proton multiplets at τ 8.10 and 6.12. Studies on the spin decoupling for these signals provided additional information regarding above groups, consistent with the presence of unit $CH_2=\underline{C}-CH_2-CH_2-O-$ in the unreduced molecule. The doublet at τ 8.90 in dihydrobicyclomycin is altered to a singlet by irradiation at τ 7.73. This result clearly indicates that this methyl proton is coupled with the proton of a -CH- group. The multiplet at τ 8.10 is changed to a broad singlet by irradiation at τ 6.12. Conversely, irradiation at τ 8.10 changed the multiplet at τ 6.12 to an AB-type quartet. This latter experiment also indicates that both methylenic protons are coupled with each other. These results account for all but three of the skeletal carbon atoms of bicyclomycin.

The presence of two amide groups in

Fig. 1. NMR spectra of bicyclomycin and dihydrobicyclomycin

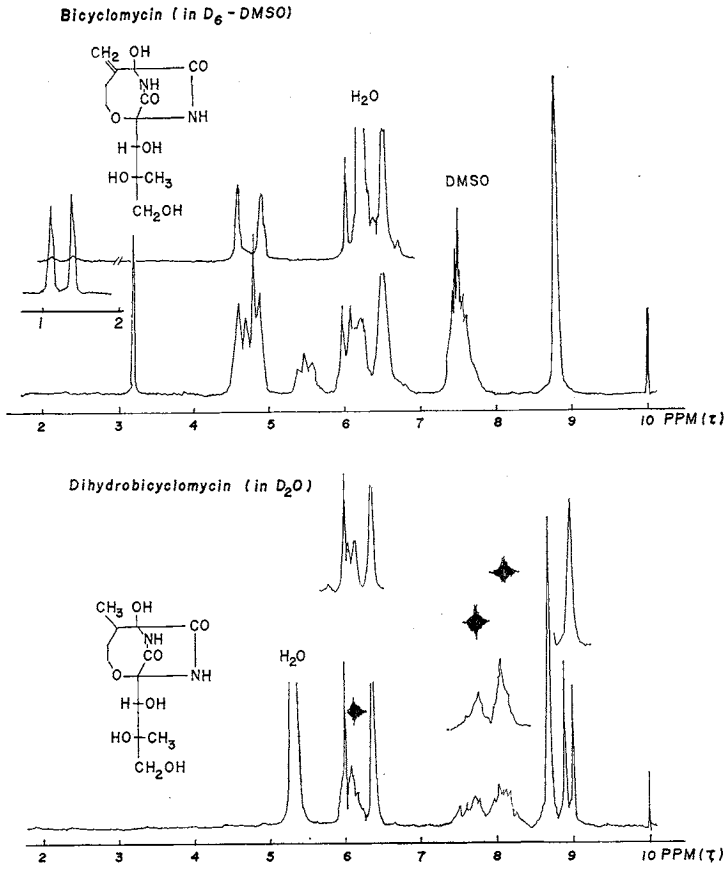
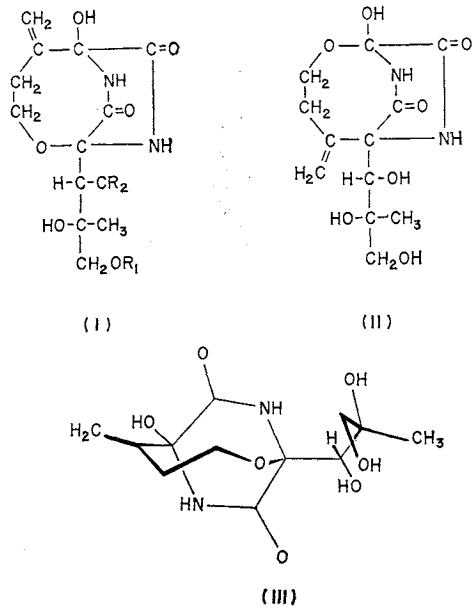


Fig. 2.



bicyclomycin was indicated by the IR-spectrum and permanganate oxidation. These amides can be assumed to be involved in a ring system, since there are strong bands at 1703 and 1673 cm^{-1} (amide I) and no band around 1550 cm^{-1} (amide II).[3] In addition, the NMR spectrum shows two signals due to -NHCO group at τ 1.40 and 1.12. By oxidation with permanganate, bicyclomycin afforded oxamide. This result indicates that there are two carbons between the two nitrogens in the molecule. These results account for all the skeletal atoms and functional groups of bicyclomycin except one hydroxy group. The remaining OH group can be assumed to be on the quaternary carbon atom.

The data reported above enable us to propose the structure I or II for bicyclomycin. To confirm the structure, it was submitted to X-ray crystallographic diffraction analysis.[4] The full structure and the relative configuration of bicyclomycin were established as 8, 10-diaza-6-hydroxy-5-methylene-1-(2'-methyl-1', 2', 3'-trihydroxy-propyl)-2-oxabicyclo [4, 2, 2] decan-7, 9-dione (I, R=R'=H) and III respectively. The absolute configuration is now under investigation.

Biological Properties

Bicyclomycin is poorly absorbed on oral administration, which may be due to its highly hydrophilic nature. The hydroxy groups in the molecule can be converted by esterification with carboxylic acids to more lipophilic products. Since a certain balance of lipo- and hydrophilic properties are required for oral absorption. A number of acyl derivatives were prepared by the usual method (see Experiment) and their biological properties were examined. All the derivatives were inactive or slightly active *in vitro*.

When the mono-acyl derivatives were given orally to rats, bicyclomycin was excreted in the urine. Data in Table 1 reveal interesting relationships existing between lipophilicity of the mono-acyl derivatives and of urinary excretion. The recovery increases with the increase in the lipophilicity of the derivatives. It is noteworthy that bicyclomycin was recovered nearly quantitatively in a 24-hour urine collection after giving benzoates or palmitate of this antibiotic. These facts clearly indicate that these acyl groups play an important role in their absorption and are readily hydrolyzed in the body.

In contrast, di-acyl derivatives were excreted unmetabolized,

Table 1. Urinary recovery of bicyclomycin after administration of acyl derivatives. (rats, 100 mg/kg p.o.)

Compounds	Structure (I)		Urine recovery(%)*
	R_1	R_2	
Bicyclomycin	H	H	24.1
a) Acetate	$-COCH_3$	H	34.2
b) Propionate	$-COCH_2CH_3$	H	51.5
c) Butyrate	$-COCH_2CH_2CH_3$	H	94.0
d) Cyclohexane carboxylate		H	100.3
e) Palmitate	$-CO(CH_2)_{14}CH_3$	H	104.5
f) Benzoate		H	80.5
g) p-Chlorobenzoate		H	113.7
h) 3,4-Dimethyl benzoate		H	98.5
i) Cinnamate		H	72.8
j) α-Furoate		H	66.7
k) Dibutyrate	$-COCH_2CH_2CH_3$	$-COCH_2CH_2CH_3$	34.3
l) Dibenzoate			0.6
m) Di α-Furoate			31.3

* Percentage recovery of bicyclomycin determined by the cup-plate method over 24 hours.

accompanied by a small amount of the antibiotic. Although the lipophilicity of di-acyl derivatives is considered to be much higher than that of mono-acyl derivatives, it is of interest that the mono-acyl derivatives, unlike the di-acyl derivatives, are readily hydrolyzed by enzymes such as esterases. These biological data will be detailed in a subsequent paper.

Experimental

Melting points were determined with a Thomas-Hoover melting point apparatus in unsealed capillary tubes and are uncorrected. IR spectra were recorded on a Hitachi Type EPI-S 2 spectrophotometer. NMR spectra and spin decoupling study were measured with a JEOL-MH-60 spectrometer in D_6-DMSO and D_2O, respectively. High resolution mass spectra were measured with a JEOL-JMS-OISG spectrometer.

Dihydrobicyclomycin: A solution of bicyclomycin (9.06 g) in water (100 ml) was shaken with Pt_2O (0.4 g) in hydrogen atmosphere at room temperature. One molar equivalent of hydrogen was absorbed in an hour and no more hydrogen was absorbed after another half an hour. The catalyst was filtered off and the filtrate was lyophilized. The residue was recrystallized from MeOH-Et_2O to give colorless prisms (6.2 g) m.p. 191~192°C.

Anal. Calcd. for $C_{12}H_{20}O_7N_2$ (304.30): C 47.36, H 6.63, N 9.21.
Found: C 47.21, H 6.74, N 8.95.

Periodic acid oxidation of bicyclomycin: Periodic acid (5.57 g) was added to a cold solution of bicyclomycin (3.02 g) in water (40 ml) and stirred for 3 hours at 0°C. This was worked up as follows:

a) Volatile products: The reaction mixture was stirred with KCl (1.5 g) and EtOH (20 ml) for 10 minutes. After filtration, the filtrate was steam distilled and *ca.* 500 ml of the distillate was collected. An aliquot of this distillate was treated with a solution of 2, 4-dinitrophenylhydrazine in H_3PO_4 and EtOH to give orange yellow needles. Its IR spectrum was superimposable with that of authentic formaldehyde 2, 4-dinitrophenylhydrazone prepared from formaldehyde by the usual method.

Another aliquot of above distillate was adjusted to pH 8.5 with 10% NaOH and evaporated to dryness *in vacuo*. The residue was dissolved in water (2 ml) and heated to reflux with *p*-bromo-phenacyl bromide (0.8 g) and EtOH (2 ml) for 1.5 hours. After cooling, the precipitate was filtered and recrystallized from aqueous EtOH to give *p*-bromophenacyl acetate as colorless needles, m.p. and mixed m.p., 83~84°C. Quantitatively, 0.77 mole equivalent of formaldehyde and 0.33 mole equivalent of acetic acid were isolated.

b) Hemiacetal: The oxidation mixture was treated with Amberite IR-45 (OH form) and evaporated to dryness *in vacuo*. The residue was extracted with hot MeOH. The extract was evaporated and the residue was recrystallized from MeOH to give colorless crystals, m.p. above 300°C.

Anal. Calcd. for $C_{10}H_{14}O_6N_2$: C 46.55, H 5.47, N 10.86, MeO 12.02.
Found: C 46.53, H 5.56, N 10.63, MeO 11.95.

IR: ν_{max}^{nujol}; 3400, 3280, 1675 cm^{-1}. NMR: τ_{ppm} in D_6-DMSO, *ca.* 7.06 (m, 2H), 6.70 (s, 3H), *ca.* 6.30 (m, 2H), 5.32 (d, J=8.0 Hz, 1H), 4.95 (broad s, 1H), 4.63 (broad s, 1H), 3.49 (d, J=8.0 Hz, 1H), 3.20 (s, 1H), 2.00 (broad s, 1H), 1.15 (broad s, 1H).

2, 4-Dinitrophenylhydrazone: m.p. 230~231°C (dec.).

Reduction of hemiacetal: A solution of the above hemiacetal (0.3 g) in MeOH (20 ml) was shaken with Pt_2O (0.05 g) in hydrogen atmosphere for 7.5 hours at room temperature. Two moles of hydrogen were absorbed. The reaction mixture was worked up as usual followed by recrystallization from EtOH, to give colorless crystals (0.2 g), m.p. 198~199°C (dec.). Mass: M^+ 230.09 ($C_9H_{14}O_5N_2$). IR: ν_{max}^{nujol}: 3400, 3220, 3110, 1695 cm^{-1}. NMR: τ_{ppm} in D_2O, 8.95 (d, J=6.6 Hz, 3H), *ca.* 8.10 (m, 2H), *ca.* 7.70 (m, 1H), 6.36 (d, J_{AB}=11.0 Hz, 1H), *ca.* 6.10

(m, 2H), 6.01 (d, J_{AB}=11.0 Hz, 1H).

Permanganate oxidation of bicyclomycin: A solution of $KMnO_4$ (1.3 g) in water (45 ml) was added dropwise to a solution of bicyclomycin (0.68 g) in acetone (45 ml) during an hour with stirring. After stirring for an additional 2 hours at 60°C, the precipitated MnO_2 was filtered off and the filtrate was treated with Dowex-50×8 (H form) (12 g). The deionized solution was evaporated to dryness *in vacuo*. By treatment with water (3 ml) the residue gave a solid substance which was purified by vacuum sublimation (~165°C/1 mmHg) to give colorless crystals (0.02 g), m.p. above 300°C. IR spectrum of this product was superimposable with that of authentic oxamide prepared from oxalyl chloride and ammonium hydroxide by the usual method.

Acyl derivatives of bicyclomycin: A typical procedure is as follows: To a stirred solution of bicyclomycin (3 g) in pyridine (9~15 ml) was added dropwise the equivalent moles of acid chloride or acid anhydride during 1~3 hours at appropriate temperature (−15~−10°C for acetate and propionate; 0~5°C for cyclohexanecarboxylate, 3, 4-dimethyl benzoate, cinnamate and α-furoate; and 25~30°C for butyrate, palmitate, benzoate, *p*-chlorobenzoate, dibutyrate, dibenzoate and di-α-furoate).

After stirring for additional 0.5~3 hours, the reaction mixture was diluted with 2~3 times volumes of cold water and evaporated *in vacuo*. The residue was taken up into AcOEt and washed with cold 5% HCl, water, cold 5% $NaHCO_3$ and water, successively. The organic layer was dried and evaporated *in vacuo* to give the crude product which was recrystallized from suitable solvents. Thus the following derivatives were prepared.

a) Acetate: m.p. 213~215°C dec. (Me_2CO).
Anal. Calcd. for $C_{14}H_{20}O_8N_2$: C 48.83, H 5.86, N 8.14.
Found: C 48.91, H 5.81, N 8.08.

b) Propionate: m.p. 178~179°C. (AcOEt).
Anal. Calcd. for $C_{15}H_{22}O_8N_2$: C 50.27, H 6.19, N 7.82.
Found: C 50.18, H 6.25, N 7.51.

c) Butyrate: m.p. 139~140°C. (AcOEt/benzene).
Anal. Calcd. for $C_{16}H_{24}O_8N_2$: C 51.60, H 6.50, N 7.52.
Found: C 51.53, H 6.58, N 7.34.

d) Cyclohexanecarboxylate: m.p. 183~185°C. dec. (AcOEt)
Anal. Calcd. for $C_{19}H_{28}O_8N_2$: C 55.33, H 6.84, N 6.79.
Found: C 55.07, H 6.86, N 6.60.

e) Palmitate: m.p. 156~157°C. (MeCN).
Anal. Calcd. for $C_{28}H_{48}O_8N_2$: C 62.20, H 8.95, N 5.18.
Found: C 62.14, H 9.17, N 5.05.

f) Benzoate: m.p. 135°C. ($AcOEt/Et_2O$).
Anal. Calcd. for $C_{19}H_{22}O_8N_2$: C 56.15, H 5.46, N 6.89.
Found: C 55.92, H 5.42, N 6.69.

g) *p*-Chlorobenzoate: m.p. 137°C. (AcOEt).
Anal. Calcd. for $C_{19}H_{21}O_8N_2Cl$: C 51.84, H 4.79, N 6.34, Cl 8.03.
Found: C 51.67, H 4.75, N 6.23, Cl 8.30.

h) 3, 4-Dimethylbenzoate: m.p. 153°C. dec. (AcOEt).
Anal. Calcd. for $C_{21}H_{26}O_8N_2$: C 58.06, H 6.03, N 6.45.
Found: C 58.30, H 6.26, N 6.32.

i) Cinnamate: m.p. 150°C. dec. ($CHCl_3/Et_2O$).
Anal. Calcd. for $C_{21}H_{24}O_8N_2$: C 58.33, H 5.59, N 6.48.
Found: C 58.16, H 5.62, N 6.20.

j) α-Furoate: m.p. 125°C. (AcOEt/benzene).
Anal. Calcd. for $C_{17}H_{20}O_9N_2$: C 51.51, H 5.09, N 7.07.
Found: C 51.32, H 5.30, N 7.11.

k) Dibutyrate: m.p. 160.5~162.5°C. (AcOEt/benzene).

Anal. Calcd. for $C_{20}H_{30}O_9N_2$: C 54.29, H 6.83, N 6.33.
Found: C 54.10, H 6.84, N 6.16.

l) Dibenzoate: m.p. 209°C. ($CHCl_3$).
Anal. Calcd. for $C_{26}H_{26}O_9N_2$: C 61.17, H 5.13, N 5.49.
Found: C 61.30, H 5.12, N 5.45.

m) Di-α-furoate: m.p. 183~184°C. ($CHCl_3$/MeOH).
Anal. Calcd for $C_{22}H_{22}O_{11}N_2$: C 53.88, H 4.52, N 5.71.
Found: C 53.66, H 4.33, N 5.53.

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