SEMISYNTHETIC BICYCLOMYCIN DERIVATIVES: PREPARATION AND ANTIBACTERIAL EVALUATION

B. W. MÜLLER, O. ZAK, W. KUMP, W. TOSCH and O. WACKER

Research Department, Pharmaceuticals Division, CIBA-GEIGY Ltd., Basle, Switzerland

(Received for publication April 16, 1979)

A number of semisynthetic bicyclomycin derivatives have been prepared by modifications at various sites of the molecule. The preparation, characterization and antimicrobial evaluation of the new compounds is described. In contrast to bicyclomycin itself, the new derivatives **48** and **58** are also active against *Proteus* species. Otherwise, the antibacterial potency of the bicyclomycin molecule was found to be very sensitive to structural changes.

The isolation of bicyclomycin[1] from *Streptomyces sapporonensis* ATCC 21532 was reported in 1972 by the research laboratories of Fujisawa Pharmaceutical Co. Ltd. The structural elucidation[2,3], antibacterial properties[4] and mechanism of action[5] have been the subjects of further communications from this group*. The antimicrobial spectrum of bicyclomycin, its low toxicity and its novel structure prompted us to initiate a project for the chemical modification of this antibiotic. The present paper describes the preparation, chemical characterization and the microbiological properties of a number of new semisynthetic derivatives obtained in our laboratories.

Chemical Modifications

A considerable number of esters of the primary hydroxyl group have been described by KAMIYA *et al.*[2,6] In extension of this work, we have now prepared the carbonates **2**, **3** and **4** by reaction of **1** with the corresponding chloroformates. One noteworthy feature in this series was the formation of the cyclic carbonate **4**, obtained with 2,2,2-trichloroethyl-chloroformate, and its rearrangement to the isomeric 1′,2′-carbonate **5** in methanolic solution at ambient temperature. The carbamate **6** was prepared easily from **1** and ethyl isocyanate.

The reaction of bicyclomycin with dihydropyrane/*p*-toluenesulfonic acid can be conducted to give either the monoether **8** or the diether **12**[7]. Acylation of **8** with benzoyl chloride - pyridine, separation of **9** from the dibenzoate **10**, and removal of the protecting group led to the C-1′-benzoate ester **11**. In a similar way, 6-O-acetyl-bicyclomycin **14** was obtained from the di-THP-ether **12** *via* the intermediate **13****. These sequences illustrate the application of THP-protected intermediates for selective transformations at C-1′ or C-6.

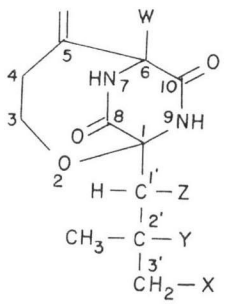
With mesyl chloride - pyridine, bicyclomycin **1** was converted to the mesylate **15**, which on treatment with triethylamine furnished the epoxide **16** in 70% yield***. Both **15** and **16** are potential intermediates for derivatives carrying a nitrogen- or sulfur functional group at C-3′. Attempts to prepare 3′-amino derivatives by reacting either **15** or **16** with ammonia or isopropylamine failed, and instead the tricyclic compound **17** was formed in low yield together with other compounds of as yet

* The absolute configuration has just recently been determined by MAAG *et al.*[13]

** Acetylation of unprotected bicyclomycin yields triacetate **7**[2,6].

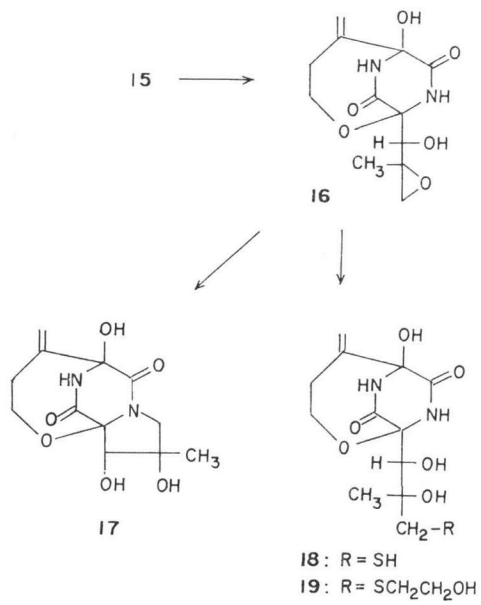
*** **16** is also accessible *via* 3′-O-tosyl-bicyclomycin[7].

Fig. 1.



No	X	Y	Z	W
1	–OH	–OH	–OH	–OH
2	$-OCOOC_2H_5$	–OH	–OH	–OH
3	$-OCOOC_2H_5$	–OH	$-OCOOC_2H_5$	–OH
4	–OCOO–		–OH	–OH
5	–OH	–O–CO–O–		–OH
6	$-OCONHC_2H_5$	–OH	–OH	–OH
7	$-OCOCH_3$	–OH	$-OCOCH_3$	$-OCOCH_3$
8	–OTHP	–OH	–OH	–OH
9	–OTHP	–OH	$-OCOC_6H_5$	–OH
10	–OTHP	–OH	$-OCOC_6H_5$	$-OCOC_6H_5$
11	–OH	–OH	$-OCOC_6H_5$	–OH
12	–OTHP	–OH	–OTHP	–OH
13	–OTHP	–OH	–OTHP	$-OCOCH_3$
14	–OH	–OH	–OH	$-OCOCH_3$
15	$-OSO_2CH_3$	–OH	–OH	–OH

Fig. 2.

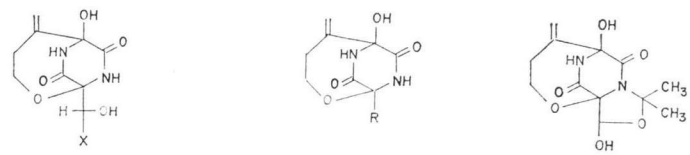


18: R = SH
19: R = SCH_2CH_2OH

unknown structure. Alternatively **17** was isolated as the main product on reaction of the epoxide **16** with NaI in aqueous solution. Opening of the oxirane ring of **16** with S-nucleophiles (H_2S and $HSCH_2CH_2OH$) did provide the desired 3′-mercapto analog of bicyclomycin (**18**) and thioether **19** respectively (Fig. 2).

As further variations of the 2-methyl-1,2,3-trihydroxy-propyl chain, its stepwise degradation and replacement by synthetic chains were envisaged. The periodic acid oxidation of bicyclomycin leading to the aldehyde **25** and the hemiacetal **26** has been described earlier[2]. We have now found that oxidation of **1** with only 1.0 eq. of periodic acid affords the methyl ketone **20**. Reduction of **20** with $NaBH_4$ led to the triol **21**, which was obtained as an epimeric mixture. Further transformations of **20** include the preparation of the oxime **22** and WITTIG reactions leading to the α,β-unsaturated esters **23** and **24**. Attempts to convert **24** to the corresponding free acid by

Fig. 3.



20 X = $-C(=O)CH_3$
21 X = $-CH(OH)CH_3$
22 X = $-C(=N-OH)CH_3$
23 X = $-C(CH_3)=CH-COOC_2H_5$
24 X = $-C(CH_3)=CH-COOCH_2C_6H_4-p-NO_2$

25 R = –CH=O
26 R = $-CH(OCH_3)-OH$
27 R = $-CH_2OH$
28 R = $-CH(OH)CH(COCH_3)_2$
29 R = $-CH(OH)CH(COOC_2H_5)_2$
30 R = $-CH=CHCOOCH_2C_6H_4-p-NO_2$
31 R = $-CH=CHCOCH_3$
32

hydrogenolytic ester cleavage were unsuccessful.

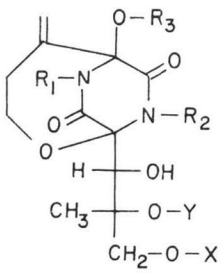
The aldehyde **25** was easily reduced with $NaBH_4$ to the primary alcohol **27**. The highly reactive carbonyl group of **25** allowed condensations with acetylacetone (→**28**) and with diethyl malonate (→**29**) in the presence of piperidine at room temperature. Attempted aldol-type condensation of **25** with acetone afforded the N,O-acetonide **32** in low yield. The olefinic compounds **30** and **31** were obtained from **25** and the corresponding phosphoranes.

Our further efforts were concentrated on the derivatization of the bicyclic nucleus. In this part of the molecule the nitrogen atoms of the dioxopiperazine ring and the exocyclic double bond were considered ideal targets for chemical transformations.

For N-alkylation studies, the acetonide **33**[7] was chosen as a suitable starting material. Methylation of **33** with CH_3I/K_2CO_3 in DMF produced **34** in moderate yield together with traces of **35**. For the preparation of **35** realkylation of the monomethyl derivative **34** was preferred, since prolonged methylation of **33** led to the formation of rearranged compounds. From this experiment a further compound could be isolated, which was characterized as the N,N,O-trimethylderivative **36**. Deprotection of the acetonides with aqueous sulfuric acid in methanol solution then gave N-monomethyl- (**37**), N,N-dimethyl- (**38**) and N,N,O-trimethyl-bicyclomycin (**39**). The N-methyl group in **37** was located at N-9, on the basis of ^{13}C-NMR spectra, which revealed a downfield shift for the signal attributed to the adjacent C-6 (84.1 ppm, compared to 81.4 ppm in **1**). Simultaneously only a minor shift (87.7 as against 87.3 ppm) was observed for the signal of C-1.

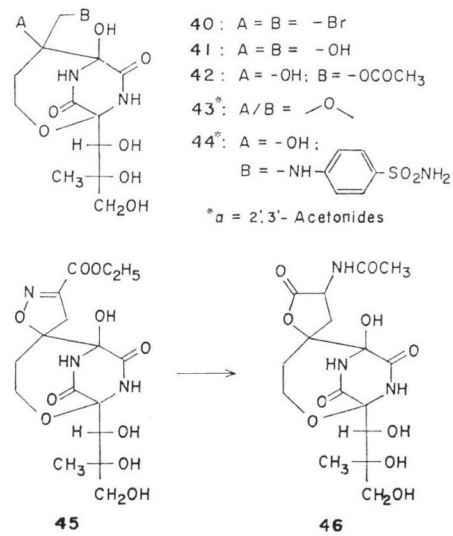
Structural changes at the 5-exo-methylene group included the preparation of the dibromo derivative **40*** with pyridinium hydrobromide perbromide and of several oxygenated compounds. Oxidation of bicyclomycin with aqueous hydrogen peroxide in the presence of osmium tetroxide in catalytic amounts afforded the hexol **41**, together with a compound resulting from oxidative degradation. With

Fig. 4.



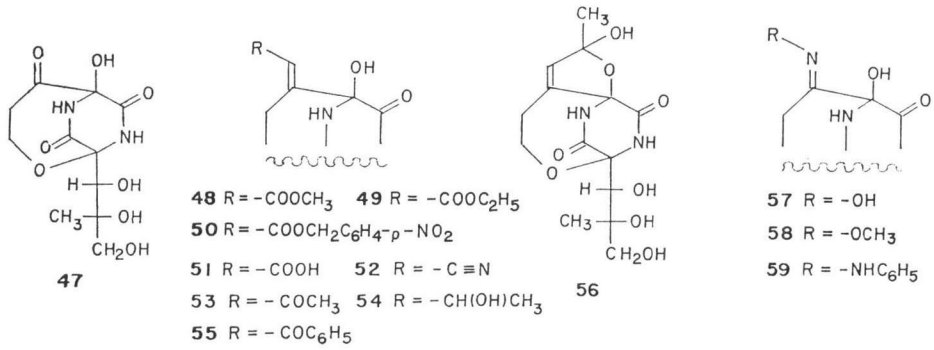
No.	$-R_1$	$-R_2$	$-R_3$	X/Y
33	-H	-H	-H	$>C(CH_3)_2$
34	$-CH_3$	-H	-H	〃
35	$-CH_3$	$-CH_3$	-H	〃
36	$-CH_3$	$-CH_3$	$-CH_3$	〃
37	$-CH_3$	-H	-H	-H/-H
38	$-CH_3$	$-CH_3$	-H	〃
39	$-CH_3$	$-CH_3$	$-CH_3$	〃

Fig. 5.



* Attempts to convert **40** into 5-aminomethyl or 5-thiomethyl derivatives failed owing to the limited stability of **40**.

Fig. 6.



sodium tungstate - hydrogen peroxide in acetic acid* both the acetate **42** (30%) and the epoxide **43** (49%) were obtained after chromatographic separation. For the epoxidation of bicyclomycin trifluoroperacetic acid was found to be the reagent of choice and gave **43** in 80% yield. According to their spectral data **41**~**43** were formed stereoselectively. Opening of the oxirane ring with sulfanilide was preferentially achieved in the 2',3'-acetonide protected series (**43a**→**44a**→**44**). Cyclo-addition of carbethoxy nitrile oxide[8] to the exocyclic double bond produced the spiro-oxazoline **45** as a single isomer. Reduction with zinc powder - acetic acid followed by mild acetylation afforded the tetracyclic lactone **46** as a mixture of two diastereomers.

Besides the addition products discussed above, we were also interested in compounds containing carbon substituents at the exocyclic double bond and in the replacement of the exo methylene group by imino functions. Both classes of compounds are accessible *via* the norketone **47**. This key intermediate was obtained in 81% yield upon ozonization of bicyclomycin followed by ozonide cleavage with dimethyl sulfide.

The α,β-unsaturated esters **48**~**50** and the nitrile **52** were prepared by WITTIG reaction of **47** with the corresponding triphenylphosphoranes. In addition **50** was converted to the carboxylic acid **51** by hydrogenolysis over Pd/C.

Attempts to prepare **53** and **55** from **47** and the triphenylphosphoranes derived from chloroacetone and chloroacetophenone respectively were unsuccessful, presumably owing to the low reactivity of the resonance-stabilized ylides and the limited thermal stability of the ketone **47**. The ketones **53** and **55** were finally obtained with the corresponding tri-*n*-butyl phosphonium ylides in dioxane solution.

According to their ^{1}H-and ^{13}C-NMR spectra, the compounds **48**~**55** were obtained as single isomers. For the methyl ester (**48**) the configuration at the trisubstituted double bond has been determined on the basis of an OVERHAUSER enhancement. Saturation of the ^{1}H-resonance attributed to the C-6 hydroxyl proton increases the NMR-intensity of the olefinic proton by 30%. This enhancement is only compatible with the sterically less crowded (E)-configuration. In addition this assignment is in good agreement with the upfield γ-shift of the ^{13}C-resonance attributed to C-4 by 6.1 ppm as compared with bicyclomycin. On the basis of this evidence and of analogous observations concerning **49**~**55**, the double bond is assumed to be of the (E)-configuration in all these compounds.

In the case of **53** further evidence for this assignment was obtained as follows: attempted reduction of the methyl ketone **53** with $NaCNBH_3$ - $CH_3NH_2 \cdot HCl$ led to the isolation of an isomeric com-

* We are indebted to Dr. T. KAMIYA for this procedure.

pound which was characterized as the hemiketal derived from the (Z)-ketone **56**. With $NaBH_4$ in methanolic solution **53** was reduced to the allylic alcohol **54**, which was obtained as a mixture of two epimers in 52% yield. The 5-imino derivatives **57**~**59** were prepared from the ketone **47** according to standard procedures. The oxime **57** and the phenylhydrazone **59** were isolated as single compounds according to ^1H- and ^{13}C-NMR, whereas the methoxyimino-derivative **58** was obtained as a 4:1 mixture of both isomers. The ^{13}C-resonance signals attributed to C-4 of **58** are located at 25.20 ppm (major component) and at 28.73 ppm (minor component). Based on the more pronounced upfield shift the (E)-configuration is assumed for the main component[9].

Biological Properties

The derivatives **2**~**59** have been screened for their antibacterial activity *in vitro* and most of them also for their efficacy in protecting mice against systemic infections.

In the *in vitro* screens, minimum inhibitory concentrations (MIC's in mcg/ml) against 25 strains of various Gram-positive and Gram-negative organisms were determined by the twofold drug-agar dilution method[10] on DST agar (Oxoid), with an inoculum of 10^4 organisms, deposited on the surface of the agar by means of a multiple replicating device[11]. By the same technique, the MIC's of a few

Table 1. Antibacterial activity *in vitro* of bicyclomycin and derivatives **2**~**46**

Organism	MIC (mcg/ml) of compound:	
	1 (bicyclomycin)	**2**~**46**
Haemophilus influenzae NCTC 4560	3.1	>100
Escherichia coli 205	12.5	>100
E. coli 205 R^+_{TEM}	12.5	>100
E. coli 16	25	>100
Salmonella typhimurium 277	25	>100
Enterobacter cloacae P99	50	>100
E. cloacae 1404	50	>100
Staphylococcus aureus 10B, *S. aureus* 2999, *Streptococcus pyogenes* Aronson, *S. faecalis* 1362/3, *S. pneumoniae* III 84, *Neisseria gonorrhoeae* 1317/4, *N. meningitidis* 1316, *Klebsiella pneumoniae* 327, *Serratia marcescens* 344, *Proteus mirabilis* 564, *P. mirabilis* 1219, *P. rettgeri* 856, *P. morganii* 2359, *P. morganii* 1518, *Pseudomonas aeruginosa* ATCC 12055, *P. aeruginosa* 313, *Clostridium perfringens* 194, *Candida albicans* ATCC 11651	>100	>100

Table 2. Efficacy of bicyclomycin and derivatives **2**~**46** against systemic infections in mice.

Organism	Route of administration	ED_{50} (mg/kg) of compound:							
		1 (bicyclomycin)	**2**	**8**	**9**	**11**	**14**	**40**	**43**
Escherichia coli 205*	s.c.	12	30	30	>300	60	170	65	200
	p.o.	110	65	150	160	100	>300	100	>300
Enterobacter cloacae P99**	s.c.	26	50	120	>300	70	n.d.	>300	n.d.

n.d.: not determined

infective dose: *4×10^6, **1×10^8 c.f.u. per mouse

All derivatives in series **2**~**46** not listed in Table 2 were ineffective against infection due to *E. coli* 205 (ED_{50} >300 mg/kg); they were not examined in infection with *E. cloacae* P99.

selected bicyclomycin derivatives for 113 clinical isolates of *Proteus* sp. received from various clinics in Europe and the U.S.A. were determined.

The protective efficacy of the derivatives was screened in mice with systemic infection due to *Escherichia coli* strain 205. Female SPF MF2 mice were infected intraperitoneally with 10 times the LD_{100} of the test organism, suspended in BHI broth with 2% mucin. Groups of 10 were then treated twice subcutaneously, immediately after infection and 3 hours later. ED_{50} values (mg/kg) were calculated by probit analysis from the number of survivors 5 days after the infection[12].

By the same technique the efficacy of some selected derivatives was also determined against systemic infections due to further bacterial genera in mice. The infecting strains and the inocula are indicated in Table 5.

The derivatives **2**~**46** were shown to be inactive *in vitro* (Table 1). Against systemic infections in mice due to *E. coli* 205 these compounds were either less active than the parent compound or even completely inactive (Table 2).

Among the 5-alkylene and 5-imino derivatives (compounds **48**~**59**), a few were found to possess a broader spectrum of activity *in vitro* than bicyclomycin (Table 3). In contrast to the parent compound, derivatives **48**, **49** and **58** also inhibited *Proteus* sp., compound **48** being the most active in this respect.

Table 3. Antibacterial activity *in vitro* of 5-alkylene and 5-imino derivatives of bicyclomycin

Organism	MIC (mcg/ml) of compound:			
	1 (Bicyclomycin)	**48**	**49**	**58**
Haemophilus influenzae NCTC 4560	3.1	>100	>100	>100
Escherichia coli 205	12.5	25	25	25
E. coli 205 R^{+}_{TEM}	12.5	25	50	50
E. coli 16	25	50	100	100
Salmonella typhimurium 277	25	50	100	50
Enterobacter cloacae P99	50	>100	>100	>100
E. cloacae 1404	50	100	>100	100
Klebsiella pneumoniae 327	25	100	>100	100
Proteus mirabilis 564	>100	100	>100	100
P. mirabilis 1219	>100	50	100	100
P. rettgeri 856	>100	25	25	>100
P. morganii 2359	>100	100	>100	>100
P. morganii 1518	>100	100	>100	>100
Pseudomonas aeruginosa ATCC 12055	>100	>100	>100	>100
Serratia marcescens 344	>100	100	>100	>100

Table 4. Activity against 113 clinical isolates of *Proteus* sp. *in vitro*

Compound	Number of strains inhibited at concentration (mcg/ml)					
	25	50	100	500	1,000	>1,000
1 (bicyclomycin)	0	0	0	0	0	113
48	1	45	34	4	0	29
58	0	3	62	17	2	29

This finding was confirmed by determining the susceptibility of 113 clinical isolates of *Proteus* sp. to compounds **48** and **58**. The methyl ester **48** inhibited 70% of these isolates at a concentration of 100 mcg/ml or less, whereas bicyclomycin did not inhibit any of the isolates even at a concentration of 1,000 mcg/ml (Table 4).

In systemic infections due to *E. coli*, *Klebsiella* sp., and *Enterobacter* sp. derivatives **48** and **58** were found to be approximately as effective as bicyclomycin. Together with compound **49**, however, they proved superior to the parent compound, displaying a marked protective effect against infections due to various strains of *Proteus* sp. (Table 5).

Table 5. Efficacy of 5-alkylene and 5-imino derivatives of bicyclomycin against systemic infection due to *Escherichia coli* 205⊕ in mice

Compound	ED_{50} (mg/kg)	
	s.c.*	p.o.*
1 (bicyclomycin)	12	102
47	>300	>300
48	18	170
49	50	>300
50	>300	>300
51	>300	>300
52	60	>300
53	>300	>300
54	>300	>300
55	>300	>300
56	>300	>300
57	55	>300
58	18	>100
59	>300	>300

⊕ infective dose 4×10^6 c.f.u. per mouse
* route of administration

Experimental Section

Bicyclomycin monohydrate was provided by Fujisawa Pharmaceutical Co. Ltd. For reactions under anhydrous conditions it was dehydrated *in vacuo* at 70°C.

Infrared spectra were obtained in nujol using a Perkin-Elmer apparatus Model 141 (main absorptions given in cm^{-1}). The UV spectra were determined on a Cary-15 spectrometer; the maxima are given in nm (ε) of λ max. The H-NMR spectra were recorded on a Varian HA-100 instrument (100 MHz) in DMSO-d_6. The signals are listed in δ values (TMS: $\delta=0.0$), J=coupling constants in Hz. Column chromatography was performed on Kieselgel 60, Merck, and for layer chromatography Merck PF 254 plates were used.

Ethyl carbonates **2** and **3**

A solution of ethyl chloroformate (4 g, 37 mmol) in THF (30 ml) was added dropwise to a stirred solution of bicyclomycin (4 g, 13 mmol) in dry pyridine (50 ml) at −10°C. The reaction mixture was then kept at room temperature for 2 hours, filtered and evaporated *in vacuo*. Column chromatography of the residue (silica gel, chloroform - methanol, 9:1) separated the reaction product into 2 components.

The compound eluted first (1.6 g, 27%) was crystallized from diethyl ether. White crystals of **3**, m.p. 197°C. $[\alpha]_D^{20}$ $-9\pm1°$ (*c* 0.718, DMSO). IR: 3500, 3320, 1770, 1745, 1700, 1675. NMR: 1.2 (t and s/9 H/CH_3), 2.2~2.8 (m/CH_2), 3.5~4.4 (m/~8H/CH_2O), 5.07 (s/HCOCOOEt), 5.37 and 5.06 (d/J=2/CH_2=C), 6.45 (s/OH), 7.0 (s/OH), 8.9 and 8.95 (s/NH). Anal. $C_{18}H_{26}N_2O_{11}$ (C,H,N).

The second component **2** (3.5 g, 70%) formed white crystals from acetone - diethyl ether, m.p. 110°C. $[\alpha]_D^{20}$ $+48\pm1°$ (*c* 0.530, DMSO). IR: 3450, 3300, 1745, 1700 (broad, unresolved). NMR: 1.22 (s/CH_3), 1.22 (t/J=7/CH_3), 2.3~2.8 (m/CH_2), 3.5~4.5 (m/CH_2O), 4.14 (q/J=7/CH_3CH_2), 5.07 and 5.40 (d/J=2/CH_2=C), 3.90 and 5.56 (AB/J=8/CHOH), 5.83 (s/OH), 6.85 (s/OH), 8.70 and 8.72 (s/NH). Anal. ($C_{15}H_{22}N_2O_9$) C,H,N.

Cyclic carbonate **4**

Bicyclomycin (3.02 g, 10 mmol) was dissolved in 35 ml of dry pyridine, cooled to −10°C and 15 ml of a dry tetrahydrofurane solution containing 1.95 ml (14.5 mmol) of 2,2,2-trichloroethyl chloroformate was added dropwise during 20 minutes with stirring. The mixture was allowed to warm to room temperature and stirring was continued for 1 hour. After evaporation *in vacuo* (35°C) the mixture could be separated into 2 compounds by silica gel column chromatography (chloroform - methanol,

9: 1). The compound eluted second was crystallized from methanol to give **5** (2.1 g, 64%). m.p. 181~183°C. IR: 3400, 3250, 1763, 1690 (broad). NMR: 1.47 (s/CH_3), 3.3~4.0 (m/CH_2O), 4.14/4.55 (AX/J=8/HCOH), 5.07 and 5.40 (d/J=2/CH_2=C), 7.0 (s/OH), 7.7 (s/NH), 9.0 (s/NH). Anal. ($C_{13}H_{16}N_2O_8$) C,H,N.

Cyclic carbonate **5**

A methanolic solution of **4** (2.0 g in 200 ml) was maintained at room temperature for 3 days. After evaporation of the solvent pure **5** crystallized from methanol as white crystals (1.0 g, 50%), m.p. 180°C. IR: 3450, 3260, 1817, 1695, 1685. NMR: 1.40 (s/CH_3), 2.2~2.8 (m/CH_2C=C), 3.5~4.2 (m/CH_2O), 5.07 and 5.40 (d/J=2/CH_2=C), 5.28 (s/HC–OCO), 7.0 (s/OH), 6.8~7.5 (broad/OH), 9.2 (s/NH), 9.7 (s/NH). MS: *m/e* 328 (M^+). Anal. ($C_{13}H_{16}N_2O_8$) C,H,N.

Ethyl carbamate **6**

Ethyl isocyanate (0.6 g, 8.5 mmol) was dissolved in THF (8 ml) and added dropwise to a stirred solution of bicyclomycin (1.2 g, 4 mmol) in dry pyridine (40 ml). After standing for 2 days at room temperature the reaction mixture was evaporated and the residue purified by column chromatography (silica gel, chloroform - acetone, 9: 1) yielding pure **6** (0.9 g, 60%), white crystals (from acetone - diethyl ether), m.p. 185~188°C, $[\alpha]_D^{20}$ +54±1° (*c* 0.728, DMSO). IR: 3550, 3350, 3230, 1680 (broad), 1670. NMR: 1.0 (t/J=7/CH_3), 1.20 (s/CH_3), 3.0 (q/J=7/CH_2), 2.3~2.6 (m/CH_2), 3.5~4.1 (m/CH_2 and CHOH) 5.04 and 5.37 (d/J=2/CH_2=C), 5.53 (d/J=8/CHOH), 6.8 and 7.0 (2H/OH), 8.7 (2H/NH). Anal. ($C_{15}H_{23}N_3O_8$) C,H,N.

3′-THP ether **8**

To a solution of **1** (22.6 g, 70.5 mmol) in dioxane (400 ml) dihydropyrane (22.4 ml, 245 mmol) and *p*-toluenesulfonic acid (0.03 g) were added. The mixture was stirred for 3 hours at room temperature, concentrated *in vacuo* and triturated with ether - petroleum ether. The resulting precipitate was isolated by filtration and then chromatographed on a short column (200 g of silica gel) with toluene - ethyl acetate (1: 1). Rotatory evaporation of the eluents and precipitation with ether gave **8** as an amorphous powder (16.2 g, 58%), m.p. 170~110°C. IR: 3415, 3255, 1690. NMR: 1.22 (s/CH_3), 1.53 (m/3×CH_2), 2.45 (m/CH_2–C=C), 3.4~3.9 (3×CH_2–O), 3.90/3.96 and 5.27/5.33 (AB/J=4/H–C–OH), 4.55 (m/O–CH–O), 5.04 and 5.37 (s/CH_2=C), 5.35 (s/OH), 6.76 (s/OH), 8.61 (s/NH), 8.77 (s/NH). Anal. ($C_{17}H_{26}N_2O_8 \cdot \frac{1}{2}H_2O$) C,H,N.

1′-O-Benzoyl-3′-THP ether **9** and 1′,6-O-dibenzoyl-3′-THP ether **10**

Benzoyl chloride (2.4 ml, 20.6 mmol) was added within 90 minutes to a solution of **8** (3.86 g, 9.75 mmol) in pyridine (15 ml). After 4 hours, the mixture was worked up with water - ethyl acetate. The organic layer was washed with water, dried with $MgSO_4$ and concentrated *in vacuo*. The remaining foam (5.3 g) was chromatographed on silica gel (120 g) whereby **10** was eluted with $CHCl_3$ - CH_3OH (97: 3). Precipitation from ligroin - ether gave **10** as an analytically pure amorphous powder (1.06 g, 17%), m.p. 135~138°C. Rf 0.80 ($CHCl_3$ - CH_3OH, 4: 1). UV (C_2H_5OH): 232 (27,200). IR: 3270, 1740, 1710. NMR: 1.29 (s/CH_3), 1.58 (m/3×CH_2), 2.69 (m/CH_2–C=C), 3.4~4.1 (m/3×CH_2–O), 4.64 (m/O–CH–O), 5.36 and 5.67 (s/CH_2=C), 5.63/5.67* (s/H–C–O), 6.23 (broad/OH), 7.4~8.3 (m/2×C_6H_5), 9.52 (s/NH), 9.59 (s/NH). Anal. ($C_{31}H_{34}N_2O_{10}$) C,H,N.

Further elution of the column with $CHCl_3$ - CH_3OH (9: 1) and crystallization from ethyl acetate afforded **9** (1.07 g, 23%), m.p. 161~165°C (dec.). Rf: 0.44 ($CHCl_3$ - CH_3OH, 4: 1). UV (C_2H_5OH): 230 (14,100). IR: 3225, 1730, 1690. NMR (DMSO-d_6): 1.21 (s/CH_3), 1.61 (m/3×CH_2), *ca.* 2.55 (m/CH_2C=C), 3.3~4.1 (m/3×CH_2–O), 4.66 (broad/O–CH–O), 5.10 and 5.44 (s/CH_2=C), 5.60/5.64* (s/HC–O), 6.07 (s/OH), 7.02 (s/OH), 7.4~8.1 (m/c_6H_5), 8.75 (s/NH), 9.29 (s/NH). Anal. ($C_{24}H_{30}N_2O_9$) C,H,N.

1′-O-Benzoate **11**

A solution of **9** (1.0 g, 2.03 mmol) in 2 ml of methanol, 2 ml of acetic acid and 1 ml of water was allowed to stand at room temperature for 24 hours and then concentrated *in vacuo*. Repeated crystallization of the residue from $CHCl_3$ - CH_3OH gave **11** (0.58 g, 70%), m.p. 185~189°C. UV (C_2H_5OH):

* double signals of the 2 diastereomers.

230 (12,950). IR: 3270, 1735, 1690 and 1670. NMR: 1.13 (s/CH_3), 2.45 (m/CH_2C=C), 3.3~3.9 (m/2×CH_2–O), 4.75 (broad/OH), 5.04 and 5.37 (d/J=1.5/CH_2=C), 5.56 (s/HC–O), 5.80 (broad/OH), 6.95, 8.69 and 9.38 (s/OH and 2×NH), 7.3~8.0 (m/C_6H_5). Anal. ($C_{19}H_{22}N_2O_8$) C,H,N.

6-O-Acetyl-1′,3′-di-THP ether **13** and 6-O-acetyl-bicyclomycin **14**

12 (8.9 g, 19.8 mmol) was acetylated with acetic anhydride (36 ml) and pyridine (36 ml) at room temperature for 20 hours. Rotatory evaporation and separation of unidentified side products by chromatography with ethyl acetate afforded **13** (3.35 g, 35%) as a white foam which was used for the next reaction without further purification.

To the above sample 60 ml of 50% aqueous acetic acid was added and the resulting solution was allowed to react at ambient temperature for 2 hours. The reaction mixture was concentrated *in vacuo* and the residue was chromatographed on 100 g of silica gel with $CHCl_3$ - CH_3OH (9: 1) to give, after recrystallization from methanol - ethyl acetate, **14** (0.71 g, 32%), m.p. ~110°C (dec.) IR: 3415, 3260, 1765, 1710, 1690. NMR (DMSO-d_6/D_2O): 1.19 (s/CH_3), 2.10 (s/CH_3C=O), 2.53 (m/CH_2C=C), 3.32/3.48 (AB/J=11/CH_2–O), 3.5~4.0 (m/CH_2–O), 3.95 (s/H–C–O), 5.20 and 5.41 (s/CH_2=C). Anal. ($C_{14}H_{22}N_2O_8$) C,H,N.

3′-O-Mesyl bicyclomycin **15**

Bicyclomycin **1** (16 g, 50 mmol) was dissolved in 150 ml of dry pyridine and at −10°C mesyl chloride (10 ml, 130 mmol) was added with stirring. The mixture was allowed to warm up to 0°C and stirred for 2 hours. After filtration the reaction mixture was evaporated *in vacuo* and pure **15** was obtained by crystallization from water (14.1 g, 74%) as white crystals, m.p. 151~153°C. IR: 3560, 3400, 3340, 3280, 1710 (broad), 1675. Anal. ($C_{13}H_{20}N_2O_9S$) C,H,N.

Epoxide **16**

A mixture of mesylate **15** (18.0 g, 47.3 mmol), triethylamine (20 g, ~200 mmol) and methanol (500 ml) was stirred at room temperature for 3 hours. Then the clear solution was evaporated and the residue was crystallized from water yielding **16** (8.6 g, 64%) as white crystals, m.p. 190~192°C. IR: 3250 (broad), 1695, 1660. NMR: 1.29 (s/CH_3), 2.2~2.8 (m/CH_2C=C), 2.64/3.07 (AB/J=6/CH_2O), 3.5~4.0 (m/CH_2O), 4.17/5.64 (AX/J=6/HCOH), 5.06 and 5.39 (d/J=2/CH_2=C), 6.9 (s/OH), 7.8 (s/NH), 8.8 (s/NH). Anal. ($C_{12}H_{16}N_2O_6$) C,H,N.

Tricyclic compound **17**

A solution of **16** (1.5 g, 5.3 mmol) and NaI (0.80 g, 5.3 mmol) in water (75 ml) was maintained at room temperature for 24 hours. After evaporation the residue was crystallized from acetone to give **17** as white prisms (1.0 g, 67%) m.p. 120°C. IR: 3440, 3230, 3100, 1690 (broad). NMR: 1.33 (s/CH_3), 2.2~2.8 (m/CH_2C=C), 3.0~4.3 (m/CH_2O), 3.29/3.54 (AB/J=12/CH_2), 4.71 (s/OH), 3.64/5.66 (AX/J=8/HCOH), 5.03 and 5.35 (d/J=2/CH_2=C), 6.82 (s/OH), 8.70 (s/NH). MS: *m/e* 284 (M^+). Anal. ($C_{12}H_{16}N_2O_6$) C,H,N.

Bicyclomycin-C-3′ thiol **18**

A solution of **16** (3.0 g, 10.6 mmol) and a few drops of triethylamine in methanol (300 ml) was saturated with H_2S during 30 minutes. After 24 hours at room temperature the reaction mixture was evaporated *in vacuo* and the residue was purified by column-chromatography (chloroform - methanol, 9: 1). From acetone **18** was obtained as white crystals (1.9 g, 57%), m.p. 183~185°C. IR: several bands between 3000 and 3500, 1695, 1675. NMR: 1.23 (s/CH_3), 2.2~3.0 (m/CH_2C=C, CH_2S), 2.0 (broad s/SH), 3.6~4.0 (m/CH_2O), 4.04/5.34 (AX/J=8/HCOH), 5.00 and 5.35 (d/J=2/CH_2=C), 5.60 (s/OH), 6.74 (s/OH), 8.60 (s/NH), 8.82 (s/NH). Anal. ($C_{12}H_{18}N_2O_6S$) C,H,N.

3′-Hydroxyethyl-thioether **19**

To a suspension of the epoxide (**16**, 1.4 g, 5 mmol) in methanol (70 ml) mercaptoethanol (3.6 ml, 5.1 mmol) was added and the reaction mixture maintained at 60°C for 7 hours under nitrogen. The reaction mixture was filtered and evaporated *in vacuo*, and the residue was chromatographed on a silica gel column (chloroform - methanol, 9: 1) to give **19** as a white amorphous solid (0.80 g, 45%). IR: 3250 (broad), 1695, 1685. NMR: 1.22 (s/CH_3), 2.5~4.0 (m/4×CH_2), 4.70 (t/J=6/CH_2OH), 5.40/4.0 (AX/J=8/HCOH), 5.04 and 5.37 (d/J=2/CH_2=C), 6.80 (s/OH), 8.64 (s/NH), 8.77 (s/NH). Anal.

($C_{14}H_{22}N_2O_7S$) C,H,N.

Methyl ketone **20**

To an aqueous solution of bicyclomycin **1** (7.6 g, 23.8 mmol, in 200 ml) H_5IO_6 (5.5 g, 24.1 mmol) was added in portions under stirring at 0°C. After 4 hours at 0°C the reaction mixture was neutralized with Amberlite IR 45 and evaporated *in vacuo*. Pure **20** was obtained by crystallization from H_2O as colourless prisms (3.7 g, 58%), decomposed above 225°C. IR: 3480, 3350, 3280, 1720, 1685, 1675. NMR: 2.23 (s/CH_3), 2.2~2.8 (m/CH_2C=C), 3.4~4.1 (m/OCH_2), 4.64/5.86 (AX/J=8/CHOH), 5.08 and 5.42 (d/J=2/CH_2=C), 6.96 (s/OH), 7.8 (s/NH), 8.9 (s/NH). MS: *m/e* 271 (M^++1). Anal. ($C_{11}H_{14}N_2O_6$) C,H,N.

Triol **21**

To a solution of the ketone **20** (540 mg, 2 mmol) in 40 ml of methanol - water (1:1) was added $NaBH_4$ (40 mg, 1.06 mmol). After 30 minutes the reaction mixture was evaporated and from the residue pure triol **21** was obtained by silica gel column-chromatography (chloroform - methanol, 9:1). Crystallization from acetone - ether yielded **21** as colorless prisms (310 mg, 57%) which decompose above 160°C and melt at about 205°C. IR: 3450, 3380, 3270, 1690 (broad). NMR: spectrum of an epimeric mixture. Anal. ($C_{11}H_{16}N_2O_6$) C,H,N.

Oxime **22**

A solution containing the ketone **20** (1.62 g, 6.0 mmol), hydroxylamine hydrochloride (0.42 g, 6.0 mmol) and pyridine (1 ml) in methanol (60 ml) was stirred at room temperature for 1 hour. After evaporation the residue was dissolved in chloroform - methanol (9:1) and passed through a silica gel column. Crystallization of the concentrated eluate from methanol - ether yielded colorless prisms of the oxime **22** (1.07 g, 63%), m.p. 133~135°C. IR: 3200 (broad), 1705, 1690. NMR: 1.84 (s/CH_3), 3.4~4.0 (m/CH_2), 4.61/5.71 (AX/J=8/HCOH), 5.07 and 5.42 (d/J=2/CH_2=C), 6.90 (s/OH), 8.04 (s/NH), 8.9 (s/NH), 11.02 (s/OH). Anal. ($C_{11}H_{15}N_3O_6$) C,H,N.

Ethyl ester **23**

A solution of the ketone (**20**, 4.32 g, 16 mmol) and ethoxycarbonylmethylene-triphenylphosphorane (5.60 g, 16 mmol) in dry dioxane (1,000 ml) was refluxed under nitrogen for 3 days. The reaction mixture was evaporated to dryness *in vacuo* and the residue was purified by silica gel column-chromatography (chloroform - methanol, 9:1). After elution of triphenylphosphine oxide pure **23** was obtained and crystallized from acetone - ether (3.0 g, 55%), m.p. 143~144°C. IR: 3540, 3420, 3200, 3100, 1720, 1695. NMR: 1.24/4.12 (t/q, J=7/C_2H_5), 2.10 (s/CH_3–C=), 2.2~2.8 (m/CH_2C=C), 3.4~4.0 (m/CH_2O), 4.68/5.73 (AX/J=6/HCOH), 5.06 and 5.40 (d/J=1.5/CH_2=C), 6.10 (s/CH=C), 6.90 (s/OH), 7.55 (s/NH), 8.80 (s/NH). Anal. ($C_{15}H_{20}N_2O_7$) C,H,N.

p-Nitrobenzylester **24**

A solution of the ketone **20** (5.4 g, 20 mmol) and *p*-nitrobenzyloxycarbonylmethylene - triphenylphosphorane (9.1 g, 20 mmol) in dry dioxane (500 ml) was maintained at 60°C for 24 hours. The reaction mixture was evaporated and the residual red oil was purified by column chromatography (chloroform - methanol, 19:1). Pure **24** was recrystallized from methanol to yield pale yellow material (3.0 g, 38%), m.p. 140°C. IR: 3480, 3370, 3230, 3100, 1700, UV (EtOH): 264 (10,900). NMR: 2.16 (s/CH_3–C=), 2.2~2.8 (m/CH_2C=C), 3.2~4.2 (m/OCH_2), 4.76/5.81 (AX/J=6/HCOH), 5.08 and 5.43 (d/J=2/CH_2=C), 5.30 (s/$CH_2C_6H_4NO_2$), 6.25 (s/–CH=), 6.92 (s/OH), 7.67 (s/NH), 7.6~8.3 (A_2B_2/J=9/C_6H_4), 8.92 (s/NH). Anal. ($C_{20}H_{21}N_3O_9$) C,H,N.

Primary alcohol **27**

A solution of bicyclomycin (19 g, 0.06 mol) in water (300 ml) was treated with H_5IO_6 (34 g, 0.15 mol) at 0°C for 2 hours. The oxidation mixture was filtered through Amberlite IR-45 (OH^--form) and the filtrate evaporated to dryness. Crude aldehyde **25** was obtained as a white solid from the residue by extraction with hot dioxane, filtration from insoluble material and evaporation (12 g, 89%)[2].

To an aqueous solution of **25** (2.26 g, 0.01 mol) in 250 ml water was added $NaBH_4$ (0.5 g, 0.013 mol) at 25°C and the mixture allowed to stand for 30 minutes. After evaporation *in vacuo* the residue was extracted with chloroform - methanol (9:1) and the resulting solution purified by filtration through

silica gel with chloroform - methanol (9:1) as eluant. Pure **27** crystallized from methyl ethyl ketone (1.37 g, 60%), m.p. 220~221°C. IR: 3490, 3380, 3200, 3080, 1693. NMR: 3.0~4.2 (m/CH_2, CH_2O), 4.88 (t/J=6/CH_2OH), 5.05 and 5.39 (d/J=2/CH_2=C), 6.8 (s/OH), 8.7 (s/2 NH). Anal. ($C_9H_{12}N_2O_5$) C,H,N.

Diketone **28**

A solution of the aldehyde **25** (6.0 g, 26.5 mmol), acetylacetone (6.0 g, 60 mmol) and piperidine acetate (0.05 g) in pyridine (70 ml) was allowed to stand at room temperature for 2 hours. The reaction mixture was evaporated to give a yellow residue which was purified by silica gel column-chromatography (chloroform - methanol, 9 : 1). Colorless crystals of **28** (2.0 g, 23%) were obtained from ethanol - ether, m.p. 186~187°C. IR: 3420, 3290, 1695. NMR: spectrum of epimeric mixture. Anal. ($C_{14}H_{18}N_2O_7$) C,H,N.

Diester **29**

A solution of the aldehyde **25** (5.0 g, 22.1 mmol), diethyl malonate (3.6 g, 22.5 mmol) and piperidine (0.05 g) in pyridine (50 ml) was allowed to stand at room temperature for 2 hours. The reaction mixture was evaporated and the residue was chromatographed (chloroform - methanol, 4: 1). Crystallization from isopropanol gave colorless crystals of **29** (1.4 g, 16%), m.p. 165~172°C. IR: 3550, 3400, 3220, 3130, 1740, 1725, 1690 (broad). NMR: 1.14 (t/J=7/CH_3), 1.16 (t/J=6/CH_3), 2.2~2.8 (m/CH_2C=C), 3.4~3.8 (m/CH_2), 3.8~4.3 (m/HCOH and OCH_2CH_3), 4.90~5.0 (dd/J=8/CH), 5.37 and 5.04 (d/J=2/CH_2=C), 5.83 (d/J=8/HCOH), 6.82 (s/OH), 8.75 (s/NH), 8.8 (s/NH). Anal. ($C_{16}H_{22}N_2O_9$) C,H,N.

p-Nitrobenzylester **30**

A solution of the aldehyde **25** (1.77 g, 7.8 mmol), *p*-nitrobenzyloxycarbonylmethylene-triphenylphosphorane (3.57 g, 7.8 mmol) in dioxane (300 ml) was maintained at 45°C for 3 hours. The reaction mixture was evaporated and from the residue the pure product **30** was obtained by silica gel column-chromatography (chloroform - methanol, 19: 1) and crystallization from acetone - ether as pale yellow prisms (0.9 g, 29%), melting between 110 and 160°C. UV (ethanol): 265 (10,200). IR: 3450, 3200, 3100, 1730, 1690. NMR: spectrum of a 2 : 1 *cis*/*trans* mixture. 6.12/6.25 (AB/J=13/CH=CH *cis*), 6.22~6.88 (AB/J=16/CH=CH *trans*). Anal. ($C_{18}H_{17}N_3O_8$) C,H,N.

Enone **31**

The aldehyde **25** (10 g, 44.2 mmol), triphenylphosphoranylidene-2-propanone (14.2 g, 44.6 mmol) and dioxane (500 ml) were maintained at 80°C for 5 hours under nitrogen. The reaction mixture was evaporated and from the residue the pure product **31** was isolated by silica gel column-chromatography (chloroform - methanol, 4: 1) followed by crystallization from aqueous ethanol (2.35 g, 20%). m.p. 209°C. IR: 3440, 3180, 3080, 1705, 1685. NMR: 2.22 (s/CH_3), 2.2~2.8 (m/CH_2C=C), 3.3~4.1 (m/CH_2O), 5.04 and 5.38 (d/J=2/CH_2=C), 6.23/6.68 (AB/J=16/CH=CH *trans*), 6.90 (s/OH), 9.0 (s/NH), 9.2 (s/NH). Anal. ($C_{12}H_{24}N_2O_5$) C,H,N.

Ketal **32**

A mixture of the aldehyde **25** (5.0 g, 22.1 mmol), dioxane (300 ml), acetone (100 ml) and Na_2CO_3·10 H_2O (1 g) was stirred at room temperature for 48 hours. The reaction mixture was filtered and evaporated *in vacuo* to give a yellow residue. Purification by silica gel column-chromatography (chloroform - methanol, 5 : 1) and crystallization from acetone gave colorless crystals of **32** (0.5 g, 7.9%), m.p. 172~173°C. IR: 3450, 3290, 3100, 1685. NMR: 1.73 (s/CH_3), 1.50 (s/CH_3), 3.4~4.1 (m/CH_2), 5.42 and 5.07 (d/J=2/CH_2=C), 5.23/7.20 (AX/J=5/HCOH), 6.97 (s/OH), 8.8 (s/NH). Anal. ($C_{12}H_{16}N_2O_6$) C,H,N.

N-Methyl-acetonide **34**

To a solution of 20.0 g (58.4 mmol) of **33** in 120 ml of DMF were added 11.0 g of anhydrous K_2CO_3 and 9.1 g (64.2 mmol) of CH_3I and the mixture was stirred at 40°C for 6 hours. The mixture was poured on 600 ml of ice-water and extracted with 8×200 ml of ethyl acetate. The extracts were washed with water, dried, and evaporated *in vacuo*. The residue (19.8 g) was chromatographed over 500 g of silica gel with $CHCl_3$ - MeOH (9: 1) and recrystallized from ether-pentane to give 8.15 g

(39.1%) of colorless crystals of **34**, m.p. 131~134°C. Rf 0.68 ($CHCl_3$ - MeOH, 9:1), IR: 3360 (sh), 3310, 1720, 1680, 1640. NMR: 1.32/1.38/1.42 ($3\times s/CH_3$), 1.98~2.60 (m/CH_2), 2.75 (s/CH_3–N), 3.53~3.85 (m/OCH_2), 3.68/4.33 (AB/J=8/CH_2O), 4.12/5.80 (AB/J=8/H–C–OH), 5.18 and 5.47 ($2\times m/CH_2$=C), 7.09 (s/OH), 8.14 (s/NH). Anal. ($C_{16}H_{24}N_2O_7$) C,H,N,O.

From other fractions a sample identified as **35*** (0.225 g, 1%) was obtained after crystallization from ether. The following compounds were obtained in substantially the same way as described above:

N,N-Dimethyl-acetonide **35**

0.56 g (18%) of colorless crystals of **35** resulted from the reaction of 3.0 g (8.41 mmol) of **34** with 1.32 g (9.25 mmol) of CH_3I in the presence of 1.6 g of anhydrous K_2CO_3 in 20 ml of DMF at 40°C for 24 hours; followed by chromatography and crystallization from ether, m.p. 182~185°C. Rf 0.75 ($CHCl_3$ - MeOH, 9:1). IR: 3330, 1700, 1670 (sh). NMR 1.12 (s/CH_3), 1.22 ($s/2\times CH_3$), 1.93~2.63 (m/CH_2), 2.73 (s/CH_3–N), 2.95 (s/CH_3–N), 3.14~3.99 (m/CH_2O), 3.73/3.98 (AB/J=9/CH_2O), 4.15/6.50 (AB/J=10/H–C–OH), 5.21 and 5.51 ($2\times m/CH_2$=C), 7.53 (s/OH). Anal. ($C_{17}H_{26}N_2O_7$) C,H,N,O.

N,N,O-Trimethyl-acetonide **36**

Thick-layer chromatography on silica gel with $CHCl_3$ - MeOH (9:1) of an additional fraction of the chromatography used for the preparation of **35**, followed by recrystallization from ether-pentane, yielded 0.32 g (10%) of white needles of **36**, m.p. 191~192°C. Rf 0.80 ($CHCl_3$ - MeOH, 9:1). IR: 3330, 1690, 1660 (sh). NMR: 1.11 (s/CH_3), 1.22 ($s/2\times CH_3$), 1.93~2.63 (m/CH_2), 2.73 (s/CH_3–N), 2.97 (s/CH_3–N), 3.13~3.99 (m/CH_2O), 3.26 (s/CH_3–O), 3.73/3.99 (AB/J=9/CH_2O), 4.18/6.22 (AB/J=10/H–C–OH), 5.27 and 5.46 ($2\times m/CH_2$=C). Anal. ($C_{18}H_{28}N_2O_7$) C,H,N,O.

N-Methyl-bicyclomycin **37**

A solution of 2.0 g (5.6 mmol) of **34** in 70 ml of MeOH and 56 ml (5.6 mmol) of 0.2 N H_2SO_4 was stirred at room temperature for 24 hours, whereafter the solution was neutralized with 75 ml of a suspension of 1.77 g (5.6 mmol) of $Ba(OH)_2\cdot 8H_2O$ in water. After the $BaSO_4$ was separated by centrifugation, the filtrate was evaporated to dryness at 30°C *in vacuo*. Chromatography of the oily residue on 60 g of silica gel with $CHCl_3$ - MeOH (9:1) and crystallization from pentane gave 1.6 g (90.3%) of hygroscopic, white crystals of **37**, melting at 88~98°C (dec.). Rf 0.2 ($CHCl_3$ - MeOH, 9:1). IR: 3400, 3300 (sh), 1730 (sh), 1680. NMR: 1.18 (s/CH_3), 1.88~2.63 (m/CH_2), 2.71 (s/CH_3–N), 3.28~3.86 ($m/2\times CH_2O$), 3.99/5.26 (AB/J=8/H–C–OH), 4.49 (broad/OH), 5.10~5.50 (m/CH_2=C/$2\times$OH), 6.96 (s/OH), 9.13 (broad/NH). Anal. ($C_{13}H_{20}N_2O_7$) C,H,N,H_2O (0.8%).

The following compounds were obtained in a similar way as described for **37**:

N,N-Dimethyl-bicyclomycin **38**

1.51 g (73.6%) of **38** resulted from the reaction of 2.3 g (6.2 mmol) of **35** with 62 ml (6.2 mmol) of 0.2 N H_2SO_4 in 70 ml of MeOH at room temperature for 6 hours, followed by chromatography and crystallization from $CHCl_3$ as a very hygroscopic, microcrystalline powder, melting at 65~80°C. Rf 0.37 ($CHCl_3$ - MeOH, 9:1). IR: 3400, 1670. NMR ($CDCl_3$): 1.12 (s/CH_3), 2.11~2.67 (m/CH_2), 2.91 (s/CH_3–N), 3.10 (s/CH_3–N), 3.19~4.09 ($m/2\times CH_2O$/OH), 4.22/5.86 (AB/J=10/H–C–OH), 5.21 and 5.66 ($2\times m/CH_2$=C), 5.40 (broad/OH), 7.28 ($s/CHCl_3$). Anal. ($C_{14}H_{22}N_2O_7$), C,H,N,H_2O (1.0%).

N,N,O-Trimethyl-bicyclomycin **39**

0.78 g (73.4%) of **39** resulted from the reaction of 1.2 g (3.12 mmol) of **36** with 31.2 ml (3.12 mmol) of 0.2 N H_2SO_4 in 60 ml of MeOH at room temperature for 15 hours, purification by thick-layer chromatography and crystallization from CH_2Cl_2 - ether as a white, microcrystalline powder, melting at 153~161°C. Rf 0.48 ($CHCl_3$ - MeOH, 9:1). IR: 3530, 3330, 1700, 1670 (sh). NMR: 1.03 (s/CH_3), 1.89~2.60 (m/CH_2), 2.69 (s/CH_3–N), 2.96 (s/CH_3–N), 3.0~4.0 ($m/2\times CH_2O$), 3.26 (s/CH_3–O), 4.13/5.47 (AB/J=10/H–C–OH), 4.29 (s/OH), 4.65 (t/J=5/CH_2–OH), 5.23 and 5.43 ($2\times m/CH_2$=C). Anal. ($C_{15}H_{24}N_2O_7$) C,H,N,O.

Dibromo bicyclomycin **40**

To a solution of **1** (5.0 g, 15.6 mmol) in dioxane (200 ml) pyridinium hydrobromide perbromide

* For further data see also preparation of **35** from **34**.

(10.0 g, 31.3 mmol) was added in small portions over a period of 6 hours. After having been stirred overnight the suspension was filtered and the filtrate was concentrated *in vacuo*. **40** was purified by chromatography ($CHCl_3$ - CH_3OH, 4: 1) and crystallization from ethyl acetate to give 4.15 g (55%) of colorless crystals, m.p. 128~133°C. IR: 3225, 1705, 1675. NMR (DMSO-d_6): 1.16 (s/CH_3), 1.8~2.8 (m/CH_2), 3.2~3.5 (m/CH_2), 3.85/5.28 (AB/J=7.5/HC–OH), 3.7~4.0 (m/CH_2), 3.98/4.24 (AB/J=12/CH_2), 4.50 (broad/OH), 5.20 (s/OH), 7.18 (s/OH), 8.84 (s/NH), 9.14 (s/NH) and signals of $CH_3COOC_2H_5$. Anal. ($C_{12}H_{18}N_2O_7Br_2 \cdot \frac{1}{4}CH_3COOC_2H_5$) C,H,N.

Oxidation of bicyclomycin with OsO_4-H_2O_2

To a solution of **1** (3.20 g, 10 mmol) and OsO_4 (64 mg, 0.25 mmol) in water (15 ml) hydrogen peroxide (~30%, 1.01 ml) was added. The mixture was stirred at ice bath temperature for 4 hours and then lyophilized. The residual foam was chromatographed on silica gel (120 g) whereby a side product (0.815 g), Rf=0.19, was eluted with $CHCl_3$ - CH_3OH (9: 1).

Further elution of the column with CH_3OH afforded, after crystallization from CH_3OH - $CH_3COOC_2H_5$, the hexol **41** (1.20 g, 36%), m.p. 180~185°C (dec.). IR: 3355, 1695, 1675. NMR: 1.16 (CH_3), 1.6~2.0 (m/CH_2), 3.2~4.1 (m/3×CH_2O), 3.85/5.10 (AB/J=7.5/HC–OH), 4.2~4.6 (m/3× OH), 5.06 (s/OH), 6.51 (s/OH), 8.10 (s/NH), 8.90 (s/NH). Anal. ($C_{12}H_{20}O_9N_2$) C,H,N.

Oxidation of bicyclomycin with Na_2WO_4-H_2O_2 to **42** and **43**.

To a solution of **1** (7.5 g, 23.4 mmol) in acetic acid (15.0 ml) and water (20 ml), Na_2WO_4 (0.25 g) and H_2O_2 (~30%, 3.0 ml) were added and the solution was stirred at ambient temperature for 18 hours. Then 0.5 ml of CH_3SCH_3 were added (KI/starch test) and the mixture was lyophilized. Repeated crystallization of the residue from CH_3OH - $CH_3COOC_2H_5$ gave the epoxide **43** (1.95 g, 26%), m.p. 194~197°C. IR: 3450, 3290, 1685. NMR: 1.17 (s), 1.6~2.0 (m/CH_2), 2.85/3.00 (AB/J=5/CH_2O), 3.3~4.0 (m/2×CH_2O), 3.94/5.23 (AB/J=7.5/HC–OH), 4.50 (t/J=5/OH), 5.15 (s/OH), 6.42 (s/OH), 8.72 (s/NH), 9.04 (s/NH). Anal. ($C_{12}H_{18}N_2O_8$) C,H,N.

Chromatography of the mother liquors with $CHCl_3$ - CH_3OH (4: 1) gave, after elution of small amounts of **43**, 2.83 g (31%) of **42**. An analytically pure sample was obtained from CH_3OH - $CH_3COOC_2H_5$, m.p. 118~122°C. IR: 3415, 3270, 1730. NMR: 1.18 (s/CH_3), 1.8~2.0 (m/CH_2), 2.03 (s/CH_3C=O), 3.3~4.4 (m/2×CH_2O), 3.88/5.18 (AB/J=7.5/HC–OH), 4.10/4.30 (AB/J=12/CH_2O), 4.46 (broad/OH), 4.97 (s/OH), 5.14 (s/OH), 7.28 (s/OH), 8.68 (s/NH), 8.96 (s/NH). Anal. ($C_{14}H_{22}N_2O_{10} \cdot H_2O$) C,H,N.

Preparation of epoxide **43** with peroxytrifluoroacetic acid

A solution of peroxytrifluoroacetic acid was prepared from 90% hydrogen peroxide (3.75 ml), trifluoroacetic anhydride (23.8 ml) and ethylene dichloride (125 ml). This reagent was added dropwise over a 45-minute period to a solution of **1** (10.0 g, 31.3 mmol) in DMF (100 ml) and ethylene dichloride (150 ml). Stirring was continued for 90 minutes and the mixture was then cooled to ice bath temperature and filtered. The crystalline residue was washed with ethylene dichloride and dried to give **43** (6.7 g, 67%). A second crop of **43** (1.3 g, 13%) was obtained from the filtrate after addition of dimethyl sulfide (1.0 ml), concentration *in vacuo* and crystallization. An analytical sample was obtained from CH_3OH - $CH_3COOC_2H_5$ and identified with **43** resulting from the oxidation with Na_2WO_4 - H_2O_2.

Acetonide **43a**

2,2-Dimethoxypropane (100 ml) and TsOH (0.10 g) were added to a suspension of **43** (10.0 g, 31.4 mmol) in acetone (200 ml) - dioxane (50 ml). The mixture was stirred at ambient temperature whereby a clear solution was obtained. After 3 hours, triethylamine (10 ml) was added and the solvents were evaporated at reduced pressure. Crystallization of the residual white foam from methanol provided **43a** (8.81 g, 78%). An analytical sample was obtained by recrystallization from acetone - ether, m.p. 190~192°C. IR: 3470, 3300, 3200, 1700, 1675. NMR: 1.25 (s/CH_3), 1.35 and 1.38 (s/CH_3–C–CH_3), 1.7~2.0 (m/CH_2), 2.55/3.05 (AB/J=5/CH_2O), 3.65/4.34 (AB/J=8/CH_2O), 3.85 (m/CH_2O), 4.01/5.78 (AB/J=8/HC–OH), 6.63 (s/OH), 8.10 (s/NH), 8.95 (s/NH). Anal. ($C_{15}H_{22}N_2O_8$) C,H,N.

Sulfonamide-acetonide **44a**

A solution of 1.43 g (4.0 mmol) of **43a** and 0.69 g (4.0 mmol) of sulfanilamide was stirred at 54°C

for 33 hours and then evaporated. Chromatography on 320 g of silica gel with $CHCl_3$ - MeOH (10:1), followed by pure MeOH, and precipitation from ether-hexane gave 1.33 g (63%) of **44a** as an hygroscopic, white powder, melting at 175~182°C (dec.). Rf 0.33 ($CHCl_3$ - MeOH, 4:1). UV (EtOH): 289 (23,600). IR: 3330, 1700. NMR: 1.28 (s/CH_3), 1.37/1.42 (2×s/CH_3), 1.87 (m/CH_2), 3.38 (broad/CH_2–NH), 3.65/4.33 (AB/J=8/CH_2O), 3.85 (m/CH_2O), 3.96/5.76 (AB/J=6/H–C–OH), 5.98 (broad/$NHCH_2$), 6.56/7.53 (A_2B_2/4H/C_6H_4–SO_2), 6.83 (broad/OH/SO_2NH_2/NH), 8.05 (s/NH). Anal. ($C_{21}H_{30}N_4O_{10}S$) C,H,N,S,H_2O (2.1%).

Sulfonamide **44**

A solution of 2.0 g (3.76 mmol) of **44a** in 75 ml of MeOH and 75.2 ml (7.52 mmol) of 0.2 N H_2SO_4 was stirred at room temperature for 16 hours, whereafter the solution was neutralized with 100 ml of a suspension of 2.37 g (7.52 mmol) of $Ba(OH)_2 \cdot 8H_2O$ in water. After the $BaSO_4$ was separated by centrifugation, the filtrate was evaporated at 30°C. Chromatography of the solid, white residue on 100 g of silica gel with $CHCl_3$ - MeOH (4:1), followed by $CHCl_3$ - MeOH (3:2), and crystallization from MeOH - ether gave 1.2 g (65%) of **44** as a very hygroscopic, microcrystalline powder, melting at 167~174°C (dec.). Rf 0.39 ($CHCl_3$ - MeOH, 3:2). UV (EtOH): 270 (21,600). IR 3420 (sh), 3300, 1700. NMR 1.06 (t/ether), 1.16 (s/CH_3), 1.86 (m/CH_2), 3.16~3.55 (m/CH_2–NH/CH_2OH/H_2O/ether), 3.77 (m/CH_2O), 3.88/5.18 (AB/J=8/H–C–OH), 4.33 (t/J=5/CH_2OH), 4.48 (s/OH), 4.82 (s/OH), 5.15 (s/OH), 6.62~7.53 (A_2B_2/4H/C_6H_5–SO_2), 6.87 (s/SO_2–NH_2), 8.22 (s/NH), 9.02 (s/NH).

Spiro isoxazoline **45**

To a solution of **1** (10.0 g, 31.3 mmol) in dioxane (300 ml) were gradually added 2-chloro-2-hydroxyimino-ethyl acetate (19.53 g, 0.129 mol) and triethylamine (19.3 ml) over a period of 95 hours. After 110 hours at room temperature the mixture was filtered and the filtrate was concentrated *in vacuo*. Trituration of the residue with ether produced a white precipitate which was separated by filtration and chromatographed ($CHCl_3$ - CH_3OH, 9:1) to give, after recrystallization from ether, the spiro isoxazoline **45** (8.2 g, 63%), m.p. 135~138°C. UV (ethanol): 244 (6,300). IR: 3425, 3290, 1670, 1605. NMR: 1.17 (s/CH_3), 1.26 (t/J=7/CH_3), 2.10 (m/CH_2), 2.87/3.63 (AB/J=18/CH_2), 3.40/3.56 (AB/J=11/CH_2), 3.82 (m/CH_2), 3.93/5.23 (AB/J=7.5/HCOH), 4.27 (q/J=7/CH_2), 4.52 (broad/OH), 5.16, 7.10, 8.90 and 9.09 (s/2×NH and 2×OH). Anal. ($C_{16}H_{23}N_3O_{10}$) C,H,N.

Lactone **46**

Zinc powder (3.5 g) was added in small portions to a solution of **45** (2.0 g, 4.8 mmol) in 25 ml of acetic acid at 20°C over 3.5 hours. After 6 hours, the mixture was filtered, the filtrate was concentrated *in vacuo* and triturated with ether to give a white powder (2.2 g). Column chromatography with $CHCl_3$ - CH_3OH (4:1) and crystallization from methanol afforded **46** (0.75 g, 38%), m.p. 220°C (dec.). IR: 3390, 3255, 1790, 1685. NMR: 1.15 (s/CH_3), 1.80/1.85* (s/CH_3CO), 2.12 (m/CH_2), 2.4~3.9 (m/3×CH_2), 3.88 and 5.16/5.19* (AB/J=7/HC–OH), 4.45 (broad/OH), 4.82 (m/CH–N), 5.09 (s/NH), 7.03/7.27* (s/OH), 8.30/8.34* (d/J=8/NH), 8.80/8.96 (s/NH), 9.06 (s/NH). Anal. ($C_{16}H_{23}N_3O_{10}$) C,H,N.

Norketone **47**

A solution of 48.0 g of bicyclomycin **1** in 2.1 liters of methanol was treated with ozone at −70°C until, after 4 hours, a blue color persisted. Dimethyl sulfide (13 ml) was added and the mixture was allowed to warm up to 5~10°C. The white precipitate was collected by filtration and a second crop was obtained on concentration of the filtrate. Recrystallization from methanol - ethyl acetate afforded **47** (37.3 g, 81%), m.p. 171~175°C (dec.). IR: 3425, 3330 and 3270, 1705, 1670. NMR: 1.18 (s/CH_3), 2.80 (m/CH_2C=O), *ca.* 3.4~4.1 (2×CH_2–O), 3.99/5.38 (AB/J=7.5/H–C–OH), 4.60 (br/OH), 5.30 (br/OH), 7.04 (s/OH), 8.98 (br/NH), 9.12 (br/NH). Anal. ($C_{11}H_{16}N_2O_8 \cdot CH_3OH$) C,H,N.

Methyl ester **48**

A solution of 18.2 g (54 mmol) of **47** and 20.0 g of carbomethoxymethylene-triphenylphosphorane in 600 ml of dioxane was kept at 70°C for 2 hours under a nitrogen atmosphere. The solvent was then removed *in vacuo* and the residue was chromatographed on 800 g of silica gel using chloroform -

* double signals, indicating the presence of 2 epimers.

methanol (4: 1) as the eluent to give, after recrystallization from water, 10.6 g (54%) of **48**, m.p. 135~136°C. IR: 3400, 3280, 1700. NMR: 1.18 (CH_3), 2.4~3.9 (m/CH_2–C=C, 2×CH_2–O), 3.62 (s/CH_3OOC), 3.91/5.23 (AB/J=8/H–C–OH), 4.48 (t/J=7/OH), 5.12 (s/OH), 6.28 (s/HC=C), 7.22, 8.77 and 9.04 (s/OH and 2×NH). Anal. ($C_{14}H_{20}N_2O_9 \cdot \frac{1}{4}H_2O$) C,H,N.

The following compounds were obtained in a similar way as described above:

Ethyl ester **49**

2.1 g (56%) of **49** resulted from the reaction of 3.04 g (10 mmol) of **47** with 3.48 g (10 mmoles) of carbethoxymethylene-triphenyl phosphorane in dioxane solution at 70°C for 2.5 hours, m.p. 116~120°C. IR: 3440, 3265, 1720, 1695. NMR: 1.18 (s/CH_3), 1.21 (t/J=7/CH_3CH_2O), 2.4~2.6 (m/CH_2–C=C and 2×CH_2–O), 3.95/5.26 (AB/J=8/H–C–OH), 4.11 (q/J=7/CH_3CH_2O), 4.50(br/OH) 5.18 (br/OH), 6.28 (s/HC=C), 7.24 (br/OH), 8.77 (s/NH), 9.04 (s/NH). Anal. ($C_{15}H_{22}N_2O_9$) C,H,N.

p-Nitrobenzyl ester **50**

The reaction of **47** (3.04 g, 10 mmol) and *p*-NO_2-benzyloxycarbonyl-methylene-triphenyl phosphorane (4.55 g, 10 mmol) gave 1.2 g (25%) of **50**, m.p. 165~169°C. IR: 3380, 1730, 1710, 1690, 1605. NMR: 1.18 (s/CH_3), 2.4~3.9 (m/CH_2C=C and 2×CH_2–O), 3.92/5.18 (AB/J=8/H–C–OH), 4.50 (br/OH), 5.20 (br/OH), 5.27 (s/CH_2), 6.38 (s/HC=C), 7.29 (s/OH), 7.63/8.22 (A_2B_2/J=9/C_6H_4), 8.80 (s/NH), 9.08. Anal. ($C_{20}H_{23}N_3O_{11} \cdot \frac{1}{2}H_2O$) C,H,N.

Carboxylic acid **51**

A solution of **50** (2.4 g, 5.0 mmol) in 100 ml of ethanol was hydrogenated over 0.12 g Pd/C. The hydrogen uptake ceased after 24 hours (473 ml, 106% of calc. volume). Filtration and evaporation of the solvent gave an oily residue which was chromatographed on silica gel (40 g). After separation of non-polar byproducts with chloroform - methanol (9: 1), **51** was eluated with chloroform - methanol (4: 1) and obtained as an amorphous solid (1.50 g, 86%) from methanol - ethyl acetate. IR: 3380, 3280, 1695. NMR: 1.18 (s/CH_3), 2.4~3.9 (m/CH_2–C=C and CH_2O), 3.34/3.47 (AB/J=11/CH_2O), 3.95 (s/H–C–O), 5.3 (br/COOH and OH), 6.27 (s/HC=C), 8.77 (s/NH), 9.04 (s/NH). Anal. ($C_{13}H_{18}N_2O_9$) C,H,N.

Nitrile **52**

The reaction of **47** (1.82 g, 6 mmol) with cyanomethylene triphenylphosphorane (1.80 g, 6 mmol) yielded **52** (0.86 g, 44%), m.p.~180°C (dec.). IR: 3350, 3250, 2225, 1730, 1705, 1625. NMR: 1.14 (s/CH_3), 2.24~3.05 (m/CH_2–C=C), 3.1~4.1 (m/2×CH_2O), 3.90/5.24 (AB/J=8/H–C–OH), 4.52 (br/OH), 5.25 (br/OH), 5.91 (s/HC=C), 7.48 (br/OH), 8.90 (br/NH), 9.10 (br/NH). Anal. ($C_{13}H_{17}N_3O_7$) C,H,N.

Acetylmethylene tri-*n*-butylphosphorane

A mixture of 34.6 ml (0.43 mol) of chloroacetone and 60 ml (0.43 mol) of tri-*n*-butyl phosphine in 1.0 liter of benzene was refluxed for 20 hours. After cooling the benzene layer was removed and the remaining viscous solid was crystallized by trituration with petroleum ether to give the hygroscopic 2-oxo-propyl-tri-*n*-butyl phosphonium chloride. Treatment of this salt with 200 ml of 2 N aqueous NaOH and 30 ml of methanol at 5°C followed by extraction with ethyl acetate and removal of the solvent afforded acetylmethylene tri-*n*-butylphosphorane as a hygroscopic resin which was used without further purification (61.0 g, 55%).

Methyl ketone **53**

A mixture of 9.06 g (27 mmol) of **47** and 11.6 g (45 mmol) of acetylmethylene tri-*n*-butylphosphorane in 300 ml of dioxane was stirred at 50°C for 5 hours. Evaporation, chromatography of the residue on silica gel with ethyl acetate - ethanol (4: 1), trituration of the concentrated fractions with cold ethyl acetate and collection by filtration gave **53** (2.17 g, 23%), m.p. 111~119°C (dec.). Rf 0.54 (chloroform - methanol, 4: 1). UV (water): 230 (8,800). IR: 3420, 3260, 1695, 1625. NMR: 1.16 (s/CH_3), 2.20 (s/CH_3CO), 2.3~4.2 (m/CH_2C=C and 2×CH_2–O), 3.95/5.28 (AB/J=8/HC–OH), 4.52 (t/J=7/OH), 5.18 (s/OH), 6.72 (s/HC=C), 7.22 (s/OH), 8.78 (s/NH), 9.06 (s/NH). Anal. ($C_{14}H_{20}N_2O_8 \cdot \frac{1}{2}H_2O$) C,H,N.

Pentol **54**

0.24 g (6.4 mmol) of $NaBH_4$ was added in small portions over a period of 15 minutes to a solution of 2.2 g (6.2 mmol) of **53** in 50 ml of methanol at 5°C. Concentration *in vacuo*, chromatography on silica gel with $CHCl_3$ - CH_3OH (1: 1) and crystallization from methanol - ethyl acetate gave 1.12 g (52%) of **54** as mixture of 2 epimers, m.p. 175~182°C. IR: 3280, 1690. NMR (DMSO/D_2O): 1.20 (s/CH_3), 1.28/1.34 (d/J=7/CH_3), 1.7~3.9 (m/3×CH_2, 2×CH), 4.92 (d/J=8/HC=C). Anal. ($C_{14}H_{22}N_2O_8 \cdot \frac{1}{2}H_2O$) C,H,N.

Phenacyl-tri-*n*-butyl phosphonium chloride

Phenacyl-tri-*n*-butyl-phosphonium chloride (56.3 g, 82.6%) resulted from the reaction of 30.92 g (0.2 Mol) of phenacyl chloride and 50.6 ml (0.2 Mol) of tri-*n*-butyl phosphine in 400 ml of ether under reflux for 21 hours. After evaporation the salt was obtained by crystallization from pentane and ether as a very hygroscopic white powder, which was used without further purification.

Phenyl ketone **55**

A mixture of 2.5 g (8.21 mmol) of **47**, 2.8 g (8.21 mmol) of phenacyl-tri-*n*-butyl-phosphonium chloride and 0.95 g (8.21 mmol) of KOtBu (97%) in 30 ml of dioxane was stirred at room temperature for 24 hours, filtered and evaporated. The oily residue was twice chromatographed over 400 and 100 g of silica gel with $CHCl_3$ - MeOH (5: 1) and ethyl acetate - MeOH (4: 1), followed by evaporation to dryness and precipitation from EtOH-pentane to give 0.73 g (22%) of **55** as a hygroscopic, amorphous powder, melting at 136~146°C. Rf 0.5 ($CH_3COOC_2H_5$ - MeOH, 4: 1). UV (EtOH): 262 (13,500). IR: 3280 (sh), 1690. NMR: 1.13 (s/CH_3), 2.90~3.86 (m/CH_2–C=CH/2×CH_2O), 3.92/5.28 (AB/J=8/H–C–OH), 4.48 (m/CH_2–OH), 5.15 (broad/OH), 7.27 (s/HC=C), 7.33 (broad/OH), 7.41~7.72 (m/3H/C_6H_5CO), 7.75~8.00 (m/2H/C_6H_5–CO), 8.87 (s/NH), 9.09 (broad/NH). Anal. ($C_{19}H_{22}N_2O_8$) C,H,N,O,H_2O (3.27%).

Hemiketal **56**

To 50 ml of dioxane were added **53** (1.3 g, 3.67 mmol), $NaCNBH_3$ (0.37 g, 5.9 mmol) and $CH_3NH_2 \cdot HCl$ (0.40 g, 5.9 mmol). The resulting mixture was allowed to react at 20°C for 18 hours. Filtration, concentration of the filtrate *in vacuo* and chromatography of the residual oil with $CHCl_3$ - CH_3OH (2: 1) afforded **58** as a white, hygroscopic powder (0.25 g, 19%), m.p. 111~119°C. IR: 3390, 3280, 1695. NMR: 1.14 (s/CH_3), 1.44 (s/CH_3), 2.4~4.2 (m/3×CH_2), 3.88/5.25 (AB/J=8/HC–OH), 4.46 (broad/OH), 5.19 (s/OH), 5.72 (s/OH), 5.78 (s/HC=C), 8.23 (s/NH), 8.96 (s/NH). Anal ($C_{14}H_{20}N_2O_8 \cdot \frac{1}{2}H_2O$) C,H,N.

Oxime **57**

To a solution of **47** (2.43 g, 8.0 mmol) in 160 ml of ethanol were added $NH_2OH \cdot HCl$ (0.56 g, 8.0 mmol) and pyridine (0.65 ml). The mixture was stirred at 60°C for 1 hour and then concentrated by rotatory evaporation. Crystallization of the residual oil from methanol - ethyl acetate gave 0.58 g of **59**. A second crop was obtained after chromatography of the mother liquid with chloroform - methanol (1: 1). Recrystallization of the combined crops gave **59** (1.84 g, 72%), m.p. 185~188°C (dec.). IR: 3500, 3330, 3240, 1705. NMR: 1.17 (s/CH_3), 2.1~2.5 (m/CH_2C=N), 3.0~3.9 (m/2×CH_2O), 3.90/5.23 (AB/J=7.5/H–C–OH), 4.47 (broad/OH), 5.17 (s/OH), 6.17 (s/OH), 8.82 (s/NH), 9.00 (s/NH), 11.47 (s/HO–N=). Anal. ($C_{11}H_{17}N_3O_8$) C,H,N.

O-Methyl oxime **58**

60 was prepared from **47** (1.21 g) and $CH_3ONH_2 \cdot HCl$ (0.34 g) by essentially the same procedure as described above for the oxime **59**. m.p. 145~148°C. IR: 3480, 3260, 1705, 1630. NMR: 1.18 (s/CH_3), 2.2~4.1 (m/3×CH_2), 3.86 (s/CH_3O), 3.95/5.33 (AB/J=8/HCOH), 4.54 (t/J=6/OH), 5.22 (s/OH), 6.52 (s/OH), 8.93 (s/NH), 9.12 (s/NH). Anal. ($C_{12}H_{19}N_3O_8$) C,H,N.

Phenylhydrazone **59**

A mixture containing **47** (2.43 g, 8.0 mmol), phenylhydrazine hydrochloride (1.16 g, 8.0 mmol) and pyridine (0.65 ml) in 160 ml of ethanol was stirred at 20°C overnight. After evaporation of the solvent the residue was purified by chromatography and crystallization from acetone - ether to give

59 (2.19 g, 68%), m.p. 165°C (dec.). UV (ethanol): 282 (16,300), 302 (13,300). IR (nujol): 3450, 3555, 1705, 1615, 1505. NMR: 1.18 (s/CH_3), 2.2~4.1 (m/3×CH_2), 3.96/5.26 (AB/J=8/HCOH), 4.50 (broad/OH), 5.18 (s/OH), 6.23 (s/OH), 6.6~7.3 (m/C_6H_5), 8.80 (s/NH), 8.98 (s/NH), 9.42 (s/NH). Anal. ($C_{17}H_{22}N_4O_7 \cdot \frac{1}{2}H_2O$) C,H,N.

Acknowledgements

The authors are indebted to Drs. H. FUHRER, G. RIST and T. WINKLER for the interpretation of the NMR spectra which proved essential for the progress of our work. We also wish to thank Dr. R. SCARTAZZINI for his support and his valuable suggestions, Dr. H. BICKEL for his interest and Prof. A. ESCHENMOSER, ETH Zürich for stimulating discussions.

References

1) MIYOSHI, T.; N. MIYAIRI, H. AOKI, M. KOHSAKA, H. SAKAI & H. IMANAKA: Bicyclomycin, a new antibiotic. I. Taxonomy, isolation and characterization. J. Antibiotics 25: 569~575, 1972
2) KAMIYA, T.; S. MAENO, M. HASHIMOTO & Y. MINE: Bicyclomycin, a new antibiotic. II. Structural elucidation and acyl derivatives. J. Antibiotics 25: 576~581, 1972
3) TOKUMA, Y.; S. KODA, T. MIYOSHI & Y. MORIMOTO: The crystal and molecular structure of bicyclomycin, a new antibiotic. Bull. Chem. Soc. Japan 47: 18~23, 1974
4) NISHIDA, M.; Y. MINE, T. MATSUBARA, S. GOTO & S. KUWAHARA: Bicyclomycin, a new antibiotic. III. *In vitro* and *in vivo* antimicrobial activity. J. Antibiotics 25: 582~593, 1972
5) TANAKA, N.; M. ISEKI, T. MIYOSHI, H. AOKI & H. IMANAKA: Mechanism of action of bicyclomycin. J. Antibiotics 29: 155~168, 1976
6) Fujisawa Pharmaceutical Co. Ltd.: Neues Antibioticum WS-4545 und dessen Derivate. Germany, Offenlegungsschrift 2,150,593.
7) KAMIYA, T.; S. MAENO & Y. KITAURA: Belgium Patent 847,475
8) SKINNER, G. S.: Evidence of the existence of aliphatic diazonium salts from the formation of chlorooximino compounds. J. Am. Chem. Soc. 46: 731~740, 1924
9) HAWKES, G. E.; K. HERWIG & J. D. ROBERTS: Nuclear magnetic resonance spectroscopy. Use of ^{13}C spectra to establish configuration of oximes. J. Org. Chem. 39: 1017~1028, 1974
10) ERICSSON, H. M. & J. C. SHERRIS: Antibiotic sensitivity testing. Acta Pathol. Microbiol. Scand. No. 217, 79B: 1~90, 1971
11) STEERS, E.; E. L. FOLZ & B. S. GARVES: An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. Antibiot. & Chemoth. 9: 307~311, 1959
12) LITCHFIELD, J. T. & F. WILCOXON: A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Therap. 96: 99~113, 1949
13) MAAG, H.; J. F. BLOUNT, D. L. COFFEN, T. V. STEPPE & F. WONG: Structure, absolute configuration, and total synthesis of an acid-catalyzed rearrangement product of bicyclomycin. J. Am. Chem. Soc. 100: 6786~6788, 1978