SEMISYNTHETIC BICYCLOMYCIN DERIVATIVES: PREPARATION
AND ANTIBACTERIAL EVALUATION

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A number of semisynthetic bicyclomycin derivatives have been prepared by modifications
at various sites of the molecule. The preparation, characterization and antimicrobial evalua-
tion 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 pro-
PERTIES 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.\(^2,3\). 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, separa-
tion 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 selec-
tive transformations at C-1' or C-6.

With mesyl chloride - pyridine, bicyclomycin 1 was converted to the mesylate 15, which on treat-
ment 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 72,6).
*** 16 is also accessible via 3'-O-tosyl-bicyclomycin\(^7\).
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 (H₂S and HSCH₂CH₂OH) 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. 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 NaBH₄ 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

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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. 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 NaBH₄ 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

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<td>(-\text{C=CH-COOCH}_2\text{H}_4\text{H}_4\text{NO}_2)</td>
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Fig. 3.
hydrogenolytic ester cleavage were unsuccessful.

The aldehyde 25 was easily reduced with NaBH₄ 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 was chosen as a suitable starting material. Methylation of 33 with CH₃I/K₂CO₃ 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-trimethyl derivative 36. Dealkylation 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 ¹³C-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.**

![Chemical structure](image)

**Fig. 5.**

![Chemical structure](image)

<table>
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<td>-CH₃</td>
<td>-CH₃</td>
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* Attempts to convert 40 into 5-aminomethyl or 5-thiomethyl derivatives failed owing to the limited stability of 40.
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** 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 1H- and 13C-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 1H-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 13C-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 NaCNBH₃ - CH₃NH₂·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 NaBH₄ 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 'H- and 13C-NMR, whereas the methoxyimino-derivative 58 was obtained as a 4:1 mixture of both isomers. The 13C-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.

**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 on DST agar (Oxoid), with an inoculum of 10⁴ organisms, deposited on the surface of the agar by means of a multiple replicating device. 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:** |
|                                 | **I** (bicyclomycin) | 2 | 46 |
| *Haemophilus influenzae* NCTC 4560 | 3.1 | >100 | >100 |
| *Escherichia coli* 205           | 12.5  | >100 | >100 |
| *E. coli* 205 R<sub>TEM</sub>    | 12.5  | >100 | >100 |
| *E. coli* 16                     | 25    | >100 | >100 |
| *Salmonella typhimurium* 277     | 25    | >100 | >100 |
| *Enterobacter cloacae* P99       | 50    | >100 | >100 |
| *E. cloacae* 1404                | 50    | >100 | >100 |

| Table 2. Efficacy of bicyclomycin and derivatives 2~46 against systemic infections in mice. |
|---------------------------------|--------|--------|--------|--------|--------|--------|--------|
| **Organism**                     | **ED₅₀ (mg/kg) of compound:** |
|                                 | **I** (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 x 10⁵, **1 x 10⁶ 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 (ED₅₀ >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 LD100 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. ED50 values (mg/kg) were calculated by probit analysis from the number of survivors 5 days after the infection123).

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

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<th>MIC (mcg/ml) of compound:</th>
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<td>1 (Bicyclomycin)</td>
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<td><em>Haemophilus influenzae</em> NCTC 4560</td>
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<td><em>Escherichia coli</em> 205</td>
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<td><em>E. cloacae</em> 1404</td>
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<td><em>Klebsiella pneumoniae</em> 327</td>
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### Table 4. Activity against 113 clinical isolates of Proteus sp. in vitro

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<th>Compound</th>
<th>Number of strains inhibited at concentration (mcg/ml)</th>
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<tr>
<td>48</td>
<td>1</td>
</tr>
<tr>
<td>58</td>
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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).

### 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-d₆. The signals are listed in δ values (TMS: δ=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. [α]D +9±1° (c 0.718, DMSO). IR: 3500, 3320, 1770, 1745, 1700, 1675. NMR: 1.2 (t and s/9 H/CH₂), 2.2-2.8 (m/CH₂), 3.5-4.4 (m/CH₂O), 5.07 (s/HCOCOEt), 5.37 and 5.06 (d/J=2/CH₂=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. C₁₃H₂₆N₂O₁₁ (C₁₄H₁₉N₂O₁₁).

The second component 2 (3.5 g, 70%) formed white crystals from acetone - diethyl ether, m.p. 110°C. [α]D +48±1° (c 0.530, DMSO). IR: 3450, 3300, 1745, 1700, 1675. NMR: 1.2 (t and s/9 H/CH₃), 2.2-2.8 (m/CH₃), 3.5-4.4 (m/8H/CH₃O), 5.07 (s/HCOCOEt), 5.37 and 5.06 (d/J=2/CH₂=C), 6.45 (s/OH), 7.0 (s/OH), 8.9 and 8.95 (s/NH). Anal. C₁₃H₂₆N₂O₁₁ (C₁₄H₁₉N₂O₁₁).

#### 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,

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<td>18</td>
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<tr>
<td>59</td>
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* route of administration

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

+ Infective dose 4×10⁶ c.f.u. per mouse
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/CH3), 3.3-4.0 (m/CH2O), 4.14/4.55 (AX/J=8/HCOH), 5.07 and 5.40 (d/J=2/CH2=C), 7.0 (s/OH), 7.7 (s/NH), 9.0 (s/NH). Anal. (C13H18N2O3) 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: 3540, 3260, 1817, 1695, 1685. NMR: 1.40 (s/CH3), 2.2-2.8 (m/CH3C=C), 3.5-4.2 (m/CH2O), 5.07 and 5.40 (d/J=2/CH2=C), 5.28 (s/HC-OCH), 7.0 (s/OH), 6.8-7.5 (broad/OH), 9.2 (s/NH), 9.7 (s/NH). MS: m/e 328 (M+). Anal. (C13H18N2O3) 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). Rotatory evaporation of the eluents and precipitation with ether - petroleum ether. The resulting precipitate was isolated by filtration and chromatographed on a short column (200 g of silica gel) with toluene - ethyl acetate (9:1). 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: 3440, 3250, 1690 (broad), 1670. NMR: 1.0 (t/J=7/CH3), 1.20 (s/CH3), 3.0 (q/J=7/CH2), 2.3-2.6 (m/CH2), 3.5-4.1 (m/CH and CHO), 5.04 and 5.37 (d/J=2/CH2=C), 5.53 (d/J=8/CHO), 6.8 and 7.0 (2H/OH), 8.7 (2H/NH). Anal. (C13H22N2O4) 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: 3440, 3250, 1690 (broad), 1670. NMR: 1.0 (t/J=7/CH3), 1.20 (s/CH3), 3.0 (q/J=7/CH2), 2.3-2.6 (m/CH2), 3.5-4.1 (m/CH and CHO), 5.04 and 5.37 (d/J=2/CH2=C), 5.53 (d/J=8/CHO), 6.8 and 7.0 (2H/OH), 8.7 (2H/NH). Anal. (C13H22N2O4) 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 MgSO4 and concentrated in vacuo. The resulting foam was chromatographed on silica gel (120 g) whereby 10 was eluted with CHCl3 - CH3OH (97:3). Further elution of the column with CHCl3 - CH3OH (9:1) and crystallization from ethyl acetate afforded 9 (1.07 g, 23%), m.p. 161-165°C (dec.). IR: 0.44 (CHCl3 - CH3OH, 4:1). UV (CH3OH): 230 (27,200). IR: 3270, 1740, 1710. NMR: 1.29 (s/CH3), 1.58 (m/3 x CH2), 2.69 (m/CH2=C-C), 3.4-4.1 (m/3 x CH2-O), 4.64 (m/O-CH-O), 5.36 and 5.67 (s/CH2=C), 5.63/5.67* (s/H-C-O), 6.23 (broad/OH), 7.4-8.3 (m/2 x CH2), 9.52 (s/NH), 9.59 (s/NH). Anal. (C13H16N2O5) C,H,N.

V-0-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 CHCl3 - CH3OH gave 11 (0.58 g, 70%), m.p. 185-189°C. UV (CH3OH):
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230 (12,950). IR: 3270, 1735, 1690 and 1670. NMR: 1.13 (s/CH$_3$), 2.45 (m/CH$_2$C=C), 3.3 ~ 3.9 (m/2 x 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 x NH), 7.3 ~ 8.0 (m/CH$_3$). Anal. (C$_{15}$H$_{22}$N$_2$O$_5$) 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$_3$OH (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$_2$O): 1.19 (s/CH$_3$), 2.10 (s/CH$_3$C--O), 2.53 (m/CH$_2$C=C), 3.32/3.48 (AB/J=11/CH$_2$-O), 3.54-4.0 (m/CH$_2$-O), 3.95 (s/H-C-O), 5.20 and 5.41 (s/CH$_2$-C). Anal. (C$_{13}$H$_{23}$N$_2$O$_6$) 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$_{31}$H$_{31}$N$_2$O$_9$S) 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$_2$C=C), 2.64/3.07 (AB/J=6/CH$_2$O), 3.5 ~ 4.0 (m/CH$_2$O), 4.17/5.64 (AX/J=8/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$_{13}$H$_{16}$N$_2$O$_6$) 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$_2$C=C), 3.0-4.3 (m/CH$_2$O), 3.29/3.54 (AB/J=12/CH$_2$-O), 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$_{13}$H$_{16}$N$_2$O$_6$) 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$_2$S 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, 1685. NMR: 1.23 (s/CH$_3$), 2.2 ~ 3.0 (m/CH$_2$C=C, CH$_3$S), 2.0 (broad s/SH), 3.6 ~ 4.0 (m/CH$_2$O), 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$_{13}$H$_{16}$N$_2$O$_6$S) 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 x CH$_2$), 4.70 (t/J=6/CH$_3$O), 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.
**Methyl ketone 20**

To an aqueous solution of bicyclomycin 1 (7.6 g, 23.8 mmol, in 200 ml) H₂IO₆ (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 H₂O as colourless prisms (3.7 g, 58%), decomposed above 225°C. IR: 3480, 3350, 3280, 1720, 1685, 1675. NMR: 2.23 (s/CH₃), 2.22.8 (m/CH₂=C), 3.4-4.1 (m/OCH₂), 4.64/5.86 (AX/J=8/CHOH), 5.08 and 5.42 (d/J=2/CH₂=C), 6.96 (s/OH), 7.8 (s/NH), 8.9 (s/NH). MS: m/e 271 (M⁺+1). Anal. (C₁₀H₁₄N₃O₅S) 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 NaBH₄ (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. (C₁₀H₁₄N₂O₆) 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 oxime 22 as colorless prisms (1.07 g, 63%), m.p. 133-135°C. IR: 3200 (broad), 1705, 1690. NMR: 1.84 (s/CH₃), 3.4-4.0 (m/CH₂), 4.61/5.71 (AX/J=8/HCOH), 5.07 and 5.42 (d/J=2/CH₂=C), 6.90 (s/OH), 8.04 (s/NH), 8.9 (s/NH). Anal. (C₁₀H₁₄N₃O₅) 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 and the residual red oil was purified by column chromatography (chloroform - methanol, 9:1). 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, 3100, 1720, 1695. NMR: 1.24/4.12 (t/q, J=7/C₂H₅), 2.10 (s/CH₂-C=), 2.22.8 (m/CH₂C=C), 3.4-4.0 (m/OCH₂), 4.76/5.81 (AX/J=6/HCOH), 5.06 and 5.40 (d/J=1.5/CH=C), 6.10 (s/CH=C), 6.90 (s/OH), 7.55 (s/NH), 8.80 (s/NH). Anal. (C₁₂H₁₀N₃O₅) C,H,N.

**p-Nitrobenzylester 24**

A solution of the ketone 20 (5.4 g, 20 mmol) and p-nitrobenzyloxycarbonylmethylene - 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 column chromatography (chloroform - methanol, 9:1). After elution of triphenylphosphine oxide pure 24 was obtained and crystallized from acetone - ether (5.4 g, 58%), m.p. 140°C. IR: 3480, 3370, 3230, 1700, UV (EtOH): 264 (10,900). NMR: 2.16 (s/CH₂-C=), 2.22.8 (m/CH₂C=C), 3.2-4.2 (m/OCH₂), 4.76/5.81 (AX/J=6/HCOH), 5.08 and 5.43 (d/J=2/CH₂=C), 5.30 (s/CH₂,C₆H₄NO₂), 6.25 (s/-CH=), 6.92 (s/OH), 7.67 (s/NH), 7.6-8.3 (A₂B₂/ J=9/C H⁺), 8.92 (s/NH). Anal. (C₁₉H₁₄N₃O₉) C,H,N.

**Primary alcohol 27**

A solution of bicyclomycin (19 g, 0.06 mol) in water (300 ml) was treated with H₂IO₆ (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%).

To an aqueous solution of 25 (2.26 g, 0.01 mol) in 250 ml water was added NaBH₄ (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/CH2, CH2O), 4.88 (t/J=6/CH2OH), 5.05 and 5.39 (d/J=2/CH2-C), 6.8 (s/OH), 8.7 (s/2 NH). Anal. (C9H12N2O3) 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. (C14H16N2O3) 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/CH3), 1.16 (t/J=6/CH3), 2.28 (m/CH2C=C), 3.4-3.8 (m/CH2), 3.84.3 (m/HCOH and OCH2CH3), 4.9-5.0 (dd/J=8/CH), 5.37 and 5.04 (d/J=2/CH2-C), 5.83 (d/J=8/HCOH), 6.82 (s/OH), 8.75 (s/NH), 8.8 (s/NH). Anal. (C16H22N2O5) 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. (C20H17N2O5) 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/CH3), 2.2-2.8 (m/CH2C=C), 3.3-4.1 (m/CH2O), 5.04 and 5.38 (d/J=2/CH2=C), 6.23/6.68 (AB/J=16/CH=CH trans), 6.90 (s/OH), 9.0 (s/NH), 9.2 (s/NH). Anal. (C18H16N2O5) C,H,N.

Ketal 32

A mixture of the aldehyde 25 (5.0 g, 22.1 mmol), dioxane (300 ml), acetone (100 ml) and Na2CO3·10 H2O (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/CH3), 1.50 (s/CH3), 3.3~4.1 (m/CH2O), 5.42 and 5.07 (d/J=2/CH2=C), 5.23/7.20 (AX/J=5/HCOH), 6.97 (s/OH), 8.8 (s/NH). Anal. (C15H18N2O6) 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 K2CO3 and 9.1 g (64.2 mmol) of CH3I 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 x 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 CHCl3 - 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 (CHCl₃ - MeOH, 9:1), IR: 3360 (sh), 3310, 1720, 1680, 1640. NMR: 1.32/1.38/1.42 (3 x s/CH₃), 1.98–2.60 (m/CH₃), 2.75 (s/CH₃-N), 3.53–3.85 (m/OCH₂), 3.68/4.33 (AB/J=8/CH₂O), 4.12/5.80 (AB/J=8/H-C-OH), 5.18 and 5.47 (2 x m/CH₂=C), 7.09 (s/OH), 8.14 (s/NH). Anal. (C₁₆H₂₄N₂O₇) 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 CH₃I in the presence of 1.6 g of anhydrous K₂CO₃ 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 (CHCl₃ - MeOH, 9:1). IR: 3330, 1700, 1670 (sh). NMR 1.12 (s/CH₃), 1.22 (s/2 x CH₃), 1.93–2.63 (m/CH₂), 2.73 (s/CH₃-N), 2.95 (s/CH₃-N), 3.14–3.99 (m/CH₂O), 3.73/3.98 (AB/J=9/CH₂O), 4.15/6.50 (AB/J=10/H-C-OH), 5.27 and 5.46 (2 x m/CH₂-C). Anal. (C₁₇H₂₆N₂O₄) C,H,N,O.

N,N,O-Trimethyl-acetonide 36

Thick-layer chromatography on silica gel with CHCl₃ - 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 0.66 (CHCl₃ - MeOH, 9:1). IR: 3330, 1690, 1660 (sh). NMR: 1.11 (s/CH₃), 1.22 (s/2 x CH₃), 1.93–2.63 (m/CH₂), 2.73 (s/CH₃-N), 2.97 (s/CH₃-N), 3.13–3.99 (m/CH₂O), 3.26 (s/CH₃-O), 3.73/3.99 (AB/J=9/CH₂O), 4.18/6.22 (AB/J=10/H-C-OH), 5.27 and 5.46 (2 x m/CH₂-C). Anal. (C₁₇H₂₆N₂O₄) 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 H₂SO₄ 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)₂·8H₂O in water. After the BaSO₄ 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 CHCl₃ - 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 (CHCl₃-MeOH, 9:1). IR: 3400, 3300 (sh), 1730 (sh), 1680. NMR: 1.18 (s/CH₃), 1.88–2.63 (m/CH₂), 2.71 (s/CH₃-N), 3.28–3.86 (m/2 x CH₂O), 3.99/5.26 (AB/J=8/H-C-OH), 4.49 (broad/OH), 5.10–5.50 (m/CH₂-C/2 x OH), 6.96 (s/OH), 9.13 (broad/NH). Anal. (C₁₃H₂₀N₂O₇) C,H,N,O (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 H₂SO₄ at room temperature for 6 hours, followed by chromatography and crystallization from CHCl₃ as a very hygroscopic, microcrystalline powder, melting at 65–80°C. Rf 0.37 (CHCl₃ - MeOH, 9:1). IR: 3400, 1700. NMR (CDCl₃): 1.12 (s/CH₃), 2.11–2.67 (m/CH₃), 2.91 (s/CH₃-N), 3.19–4.09 (m/2 x CH₂O/OH), 4.22/5.86 (AB/J=10/H-C-OH), 5.21 and 5.66 (2 x m/CH₂-C), 5.40 (broad/OH), 7.28 (s/CHCl₃). Anal. (C₁₇H₂₄N₂O₇) C,H,N,O (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 H₂SO₄ in 60 ml of MeOH at room temperature for 15 hours, purification by thick-layer chromatography and crystallization from CHCl₃ as a white, microcrystalline powder, melting at 153–161°C. Rf 0.48 (CHCl₃ - MeOH, 9:1). IR: 3530, 3330, 1700, 1670 (sh). NMR: 1.03 (s/CH₃), 1.89–2.60 (m/CH₂), 2.69 (s/CH₃-N), 2.96 (s/CH₃-N), 3.0–4.0 (m/2 x CH₂O), 3.26 (s/CH₃-O), 4.13/5.47 (AB/J=10/H-C-OH), 4.29 (s/OH), 4.65 (t/J=5/CH₃-OH), 5.23 and 5.43 (2 x m/CH₂-C). Anal. (C₁₇H₂₄N₂O₇) 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
(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 (CHCl₃ - CH₃OH, 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-d₆): 1.16 (s/CH₃), 1.8-2.8 (m/CH₂), 3.2-3.5 (m/CH₂), 3.85/5.28 (AB/J=7.5/HC-OH), 3.7-4.0 (m/CH₂), 3.98/4.24 (AB/J=12/CH₂), 4.50 (broad/OH), 5.20 (s/OH), 7.18 (s/OH), 8.84 (s/NH), 9.14 (s/NH) and signals of CH₃COOC₂H₅.

Oxidation of bicyclomycin with OsO₄-H₂O₂

To a solution of 1 (3.20 g, 10 mmol) and OsO₄ (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 CHCl₃ - CH₃OH (9:1).

Further elution of the column with CH₃OH afforded, after crystallization from CH₂OH - CH₃COOCH₃, the hexol 41 (1.20 g, 36%), m.p. 180-185°C (dec.). IR: 3355, 1695, 1675. NMR: 1.16 (CH₃), 1.6-2.0 (m/CH₂), 2.85/3.00 (AB/J=5/CH₂O), 3.3-4.0 (m/3 x CH₂O), 3.84/5.10 (AB/J=7.5/HC-OH), 4.24.6 (m/3 x OH), 5.06 (s/OH), 6.51 (s/OH), 8.10 (s/NH), 8.90 (s/NH). Anal. (C₁₂H₁₅N₂O₇) C,H,N.

Oxidation of bicyclomycin with Na₂WO₄-H₂O₂ 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), Na₂WO₄ (0.25 g) and H₂O₂ (~30 %, 3.0 ml) were added and the solution was stirred at ambient temperature for 18 hours. Then 0.5 ml of CH₃SCH₃ were added (KI/starch test) and the mixture was lyophilized. Repeated crystallization of the residue from CH₃OH - CH₃COOCH₃ gave the epoxide 43 (1.95 g, 26 %), m.p. 194-197°C. IR: 3450, 3290, 1730. NMR: 1.17 (s), 1.6-2.0 (m/CH₂), 2.55/3.05 (AB/J=5/CH₂O), 3.65/4.34 (AB/J=8/CH₂O), 3.85 (m/CH₂O), 4.10/4.30 (AB/J=8/HC-OH), 4.46 (broad/OH), 4.97 (s/OH), 5.14 (s/OH), 5.15 (s/OH), 7.28 (s/OH), 8.68 (s/NH), 8.96 (s/NH). Anal. (C₁₃H₁₈N₂O₇.H₂O) 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 CH₃OH - CH₃COOCH₃ and identified with 43 resulting from the oxidation with Na₂WO₄-H₂O₂.

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: 1.18 (s/CH₃), 1.35 and 1.38 (s/CH₃-C-CH₃), 1.7-2.0 (m/CH₂), 2.55/3.05 (AB/J=5/CH₂O), 3.65/4.34 (AB/J=8/CH₂O), 3.85 (m/CH₂O), 4.01/5.78 (AB/J=8/HC-OH), 6.63 (s/OH), 8.01 (s/NH), 8.95 (s/NH). Anal. (C₁₃H₂₂N₂O₁₇.H₂O) 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 CHCl₃ - 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 (CHCl₃ - MeOH, 4:1). UV (EtOH): 289 (23,600). IR: 3330, 1700. NMR: 1.28 (s/CH₃), 1.37/1.42 (2 x s/CH₃), 1.87 (m/CH₂), 3.38 (broad/CH₂-NH), 3.65/4.33 (AB/J=8/CH₂O), 3.85 (m/CH₂O), 3.96/5.76 (AB/J=6/H-C-OH), 5.98 (broad/NHCH₂), 6.56/7.53 (A₂B₂/4H/C₆H₄-SO₂), 6.83 (broad/OH/SO₂-NH_/NH), 8.05 (s/NH). Anal. (C₂,H₃ON₄O₅S) C,H,N,S,H₂O (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 H₂SO₄ 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•8H₂O in water. After the BaSO₄ was separated by centrifugation, the filtrate was evaporated at 30°C. Chromatography of the solid, white residue on 100 g of silica gel with CHCl₃ - MeOH (4:1), followed by CHCl₃ - 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.). UV (EtOH): 270 (21,600). IR: 3420 (sh), 3300, 1700. NMR: 1.06 (t/ether), 1.16 (s/CH₃), 1.86 (m/CH₂), 3.16-3.55 (m/CH₂-NH/CH₂OH/H₂O/ether), 3.77 (m/CH₂), 3.88/5.18 (AB/J=8/H-C-OH), 4.33 (t/J=5/CH₂OH), 4.48 (s/OH), 4.82 (s/OH), 5.15 (s/OH), 6.62~7.53 (A₂B₂/4H/C₆H₄-SO₂), 6.87 (s/SO₂-NH_), 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 (CHCl₃ - CH₃OH, 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/CH₃), 1.26 (t/J = 7/CH₃), 2.10 (m/CH₂), 2.87/3.63 (AB/J = 18/CH₂), 3.40/3.56 (AB/J = 11/CH₂), 3.82 (m/CH₂), 3.93/5.23 (AB/J = 7.5/HCOH), 4.27 (q/J = 7.5/CH₂), 4.52 (broad/OH), 5.16, 7.10, 8.90 and 9.09 (s/2 x NH and 2 x OH). Anal. (C₁₂H₂₂N₃O₆) 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 CHCl₃ - CH₃OH (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/CH₃), 1.20 (t/J = 7/CH₃), 2.10 (m/CH₂), 2.87/3.63 (AB/J = 18/CH₂), 3.40/3.56 (AB/J = 11/CH₂), 3.82 (m/CH₂), 3.93/5.23 (AB/J = 7.5/HCOH), 4.27 (q/J = 7/CH₂), 4.52 (broad/OH), 5.16, 7.10, 8.90 and 9.09 (s/2 x NH and 2 x OH). Anal. (C₁₂H₂₂N₃O₆) 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/CH₃), 2.80 (m/CH₂C=O), ca. 3.4~4.1 (2 x CH₂-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. (C₁₅H₁₄O₄S•CH₃OH) 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 -
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 (CH₃), 2.4–3.9 (m/CH₂=C=C and 2x CH₂-O), 3.62 (s/CH₃OOO), 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 2x NH). Anal. (C₁₃H₂₂N₂O₈·1/₂H₂O) 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 carboxy-methylene-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/CH₃), 1.21 (t/J=7/CH₃CH₂O), 2.4–3.6 (m/CH₂-C=C and 2x CH₂-O), 3.95/5.26 (AB/J=8/H-C-OH), 4.11 (q/J=7/CH₃CH₂O), 4.50(br/OH), 5.18 (br/OH), 5.20 (br/OH), 6.27 (s/HC=C), 7.24 (br/OH), 8.77 (s/NH), 9.04 (s/NH). Anal. (C₁₅H₂₂N₂O₉) C,H,N.

**p-Nitrobenzyl ester 50**

The reaction of 47 (3.04 g, 10 mmol) and p-NO₂-benzoylcarbonyl-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/CH₃), 2.4–3.9 (m/CH₂=C=C and 2x CH₂-O), 3.95/5.26 (AB/J=8/H-C-OH), 4.11 (q/J=7/CH₃CH₂O), 4.50(br/OH), 5.20 (br/OH), 5.27 (s/CH₃), 6.38 (s/HC=C), 7.29 (s/OH), 7.63/8.22 (A₂B₂/J=9/C,H₂), 8.80 (s/NH), 9.08. Anal. (C₂₀H₂₃N₃O₁₁·ZH₂O) 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 eluted 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/CH₃), 2.4–3.9 (m/CH₂=C=C and 2x CH₂-O), 3.34/3.47 (AB/J=11/CH₃O), 3.95 (s/H-C-OH), 5.3 (hr/COOH and OH), 6.72 (s/HC=C), 7.87 (s/NH), 9.04 (s/NH). Anal. (C₁₃H₁₉N₂O₈·1H₂O) 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/CH₃), 2.24–3.05 (m/CH₂=C=C and 2x CH₂-O), 3.1–4.1 (m/2x CH₂-O), 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. (C₁₃H₁₁N₇O₉) 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-butylphosphine in 1 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/CH₃), 2.20 (s/CH₂CO), 2.3–4.2 (m/CH₂=C and 2x CH₂-O), 3.95/5.28 (AB/J=8/H-C-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. (C₁₅H₂₆N₂O₆·1/₂H₂O) C,H,N.
Pentol 54

0.24 g (6.4 mmol) of NaBH₄ 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 CHCl₃ - CH₃OH (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/D₂O): 1.20 (s/CH₃), 1.28/1.34 (d/J=7/CH₃), 1.7-3.9 (m/3 x CH₂, 2 x CH), 4.92 (d/J=8/HC-C). Anal. (C₁₄H₂₂N₂O₈·½H₂O) 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 CHCl₃ - 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 (CH₃COCH₂ - MeOH, 4: 1). UV (EtOH): 262 (13,500). IR: 3280 (sh), 1690. NMR: 1.13 (s/CH₃), 2.90-3.86 (m/CH₂-C=CH/2 x CH₂O), 3.92/5.28 (AB/J=8/H-C-OH), 4.48 (broad/OH), 7.27 (s/HC=C), 7.33 (broad/OH), 7.41-7.72 (m/3H/CH₂CO), 7.75-8.00 (m/2H/C₆H₅-CO), 8.87 (s/NH), 9.09 (broad/NH). Anal. (C₁₃H₂₂N₂O₈) C, H, N, O, H₂O (3.27%).

Hemiketal 56

To 50 ml of dioxane were added 53 (1.3 g, 3.67 mmol), NaCNBH₃ (0.37 g, 5.9 mmol) and CH₂NH₂•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 CHCl₃ - CH₃OH (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/CH₃), 1.44 (s/CH₃), 2.4-4.2 (m/3 x CH₂), 3.88/5.25 (AB/J=8/H-C-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. (C₁₄H₂₇N₂O₈.1H₂O) C, H, N.

Oxime 57

To a solution of 47 (2.43 g, 8.21 mmol) in 160 ml of ethanol were added NH₂OH-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, 3320, 1705. NMR: 1.17 (s/CH₃), 2.1-2.5 (m/CH₃C=N), 3.0-3.9 (m/2 x CH₂O), 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). Anal. (C₁₁H₁₅N₃O₃) C, H, N.

O-Methyl oxime oxime 58

60 was prepared from 47 (1.21 g) and CH₂ONH₂•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/CH₃), 2.2-4.1 (m/3 x CH₂), 3.86 (s/CH₂O), 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. (C₁₂H₁₇N₃O₅) C, H, N.

Phenydihydrazone 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
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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/CH₃), 2.24.1 (m/3 x CH₂), 3.96/5.26 (AB/J=8/HCOH), 4.50 (broad/OH), 5.18 (s/OH), 6.23 (s/OH), 6.67.3 (m/C₈H₅), 8.80 (s/NH), 8.98 (s/NH), 9.42 (s/NH).

Anal. (C₁₇H₂₂N₄O₇.H₂O) C,H,N.

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