Preparation and Antitumor Activity of Olivomycin A Analogues

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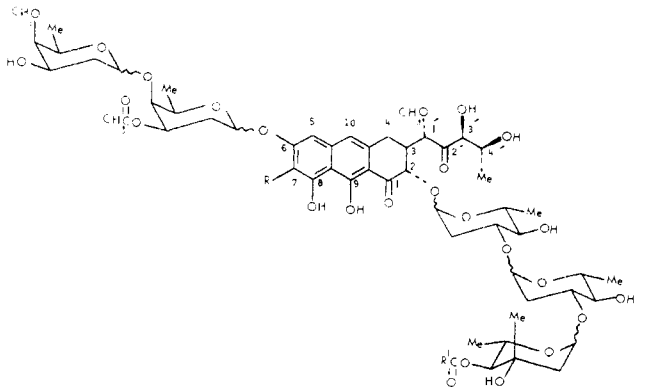
Novel analogues of olivomycin A were prepared by selective reactions involving the carbonyl and hydroxyl groups of the aglycon moiety. Electrophilic substitution of the aglycon also was successful. Of 11 analogues, all but two were active in the P-388 murine leukemia assay. One compound, the 2′-methoxime, showed superior activity to olivomycin A based on its wider dose range and greater potency. The methyl imine and the 8-*O*-methyl ether were equal to olivomycin A in potency and efficacy. Most of the other analogues were slightly less potent or effective.

The aureolic acid family of antitumor antibiotics includes three members, mithramycin (aureolic acid), chromomycin A_3, and olivomycin A, that are used for cancer chemotherapy in various parts of the world. However, these compounds are highly toxic, especially to the gastrointestinal tract, and their clinial antitumor spectrum is limited.[1,2] In the United States, only mithramycin is approved. Its use is limited to testicular cancer and certain conditions of hypercalcemia.[3]

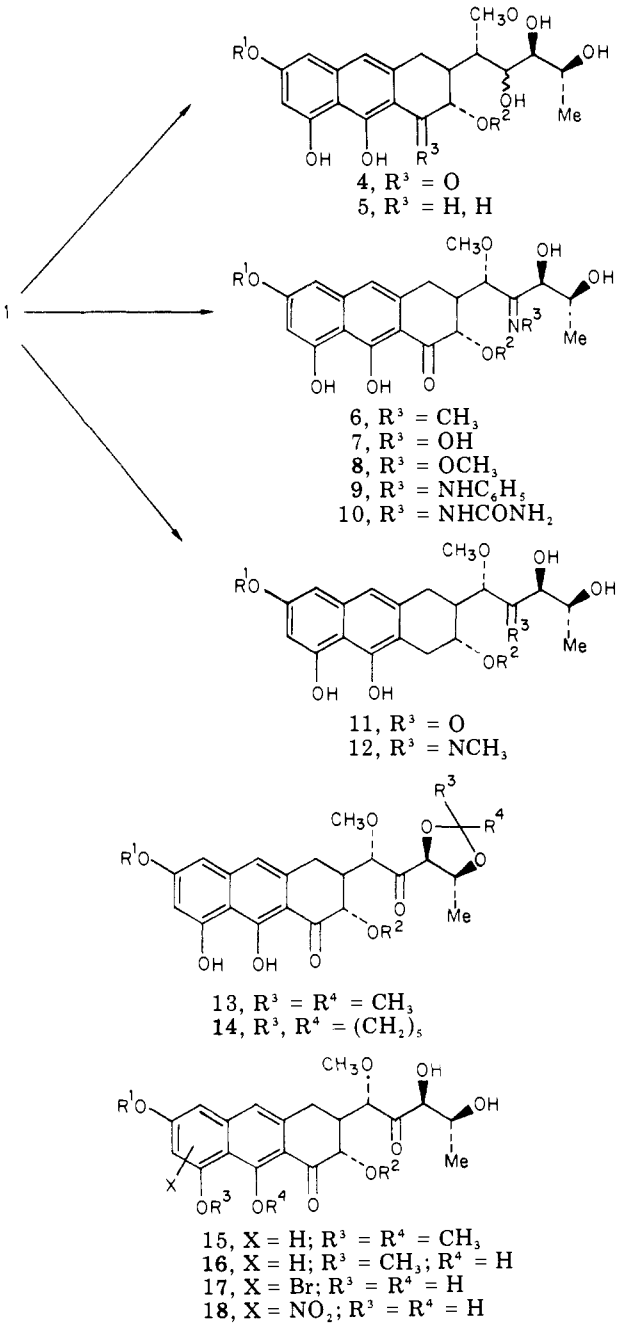
In most antitumor antibiotic families, extensive efforts have been made to prepare derivatives and analogues that might show improved therapeutic properties. However, no systematic effort has been made for the aureolic acids. The known structure–activity relationships are limited to the naturally occurring compounds and a few products of partial glycoside hydrolysis.[4] In addition, it was reported recently that the isobutyryl groups of olivomycin A (1) and



1, R = H; R^1 = $CH(CH_3)_2$
2, R = Me; R^1 = $CH(CH_3)_2$
3, R = Me; R^1 = Me

chromomycin A_2 (2) and the acetyl group of chromomycin A_3 (3) all were cleaved upon incubation with *Whetzelinia sclerotiorum*. The products maintained their antitumor activity.[5] Thus, it is apparent that certain transformations in the sugars of these compounds are compatible with activity. Concerning the aglycon, the only structural change ever noted in the intact antibiotics is that the 7-methyl group is present in chromomycins and mithramycin but not in olivomycins. We decided to investigate first the preparation of new analogues with changes in the aglycon moiety, utilizing olivomycin A (1) available from Bristol Laboratories.

Scheme I[a]



4, R^3 = O
5, R^3 = H, H

6, R^3 = CH_3
7, R^3 = OH
8, R^3 = OCH_3
9, R^3 = NHC_6H_5
10, R^3 = $NHCONH_2$

11, R^3 = O
12, R^3 = NCH_3

13, R^3 = R^4 = CH_3
14, R^3, R^4 = $(CH_2)_5$

15, X = H; R^3 = R^4 = CH_3
16, X = H; R^3 = CH_3; R^4 = H
17, X = Br; R^3 = R^4 = H
18, X = NO_2; R^3 = R^4 = H

[a] R^1 and R^2 correspond to the carbohydrate chains depicted in structure 1.

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In view of the structural complexity of olivomycin A, we were uncertain that chemical reactions could be done with any selectivity. However, it was soon apparent that selectivity was possible. In fact, most reactions gave only one product. The side-chain (2′) carbonyl group was more susceptible to nucleophiles than the 1-carbonyl group. It was selectively reduced to the alcohol **4** by sodium borohydride and it gave typical carbonyl derivatives, such as the methyl imine **6**, oxime **7**, methoxime **8**, phenylhydrazone **9**, and semicarbazone **10**, in high yields (Scheme I). The 1-carbonyl group, which is benzylic, was selectively reduced to give the corresponding 1-deoxy compound **11** by catalytic hydrogenation. Further transformations of **11** included sodium borohydride reduction of the 2′-carbonyl group to give **5** and formation of the methyl imine derivative **12**.

Treatment of olivomycin A (**1**) with excess diazomethane afforded the 8,9-bis(*O*-methyl) derivative **15**. This result contrasts with a literature report that chromomycinone (the isolated aglycon) is methylated only on the 9-hydroxyl group by diazomethane.[6] We found that treatment of olivomycin A with the dimethyl acetal of *N*,*N*-dimethylformamide gave the 8-*O*-methyl derivative **16**. This position of methylation was clearly shown by the ^{1}H NMR spectrum, which lost the peak at δ 9.6 but retained the peak at δ 15.5. The 3′,4′-*O*-isopropylidene derivative **13** of olivomycin A was obtained by stirring at 25 °C with 2,2-dimethoxypropane and *p*-toluenesulfonic acid, and the corresponding cyclohexylidene derivative **14** was similarly prepared. These derivatives and the *O*-methyl ethers **15** and **16** are important for structure–activity relationships because the complexation of aureolic acid compounds with magnesium or some other divalent metal is considered to be essential to their binding with DNA.[7]

Substituents on the 7 position of olivomycin A appeared important for antitumor activity because a methyl group is present at this position in chromomycin A_3. Treatment of olivomycin A with pyridine perbromide gave a major product and an unstable minor product. The major product had lost one hydrogen on an aromatic ring according to its ^{1}H NMR spectrum. This substitution probably took place at either the 5 or 7 position because the 10 position is in a ring that is less electron rich. However, we were unable to make a unique assignment. An attempt to determine the remaining hydrogen by NMR shifts induced by formation of the phenolate anion[8] failed because of decomposition. Tetranitromethane converted olivomycin A into the monitro derivative **18**. The position of substitution in this derivative also is uncertain, although this reagent usually nitrates ortho to the phenolic hydroxyl group.

Attempted transformations of olivomycin A that were unsuccessful included methyl ether cleavage with boron trichloride and potassium nitrosodisulfonate oxidation. Although not conclusively established, it appeared by considerable changes in TLC properties that the products had undergone sugar cleavage.

Biological Activity. Table I summarizes the antileukemia activity of the olivomycin A analogues in the standard P-388 assay in mice. Every analogue, except the semicarbazone **10** and the nitro derivative **17**, was active

Table I. Effect of Olivomycin A Derivatives on P-388 Leukemia[a]

no.	dose, ip, (mg/kg)/inj	effect MST, % T/C	av wt change, g	survivors day 5
1[b]	4	157	−0.4	6/6
	2	146	0	6/6
	1	130	+0.3	6/6
	0.5	114	+1.0	6/6
4	16	130	−0.9	6/6
	8	140	−0.2	6/6
	4	125	+0.2	6/6
	2	90	+1.1	6/6
5	16	139	+0.5	6/6
	8	106	+3.5	6/6
	4	100	+3.7	6/6
	2	100	+5.3	6/6
6	8	tox	tox	1/6
	4	155	−2.4	6/6
	2	140	+0.1	6/6
	1	122	+1.1	6/6
7	4	tox	tox	3/6
	2	155	−0.8	6/6
	1	100	+0.8	6/6
	0.5	100	+1.3	6/6
8	8	144	−2.8	4/6
	4	156	−1.3	4/6
	2	167	+0.3	6/6
	1	144	+0.9	6/6
	0.5	156	+0.8	6/6
	0.25	128	+1.3	6/6
9	16	140	−1.3	6/6
	8	140	−0.5	6/6
	4	120	+0.5	6/6
	2	120	+0.2	6/6
10	16	105	−2.3	6/6
	8	110	−1.2	6/6
	4	110	−1.3	6/6
	2	85	+1.8	6/6
11	8	150	−1.0	4/6
	4	140	−0.7	6/6
	2	150	−0.7	6/6
	1	110	+1.1	6/6
12	8	115	−3.3	4/6
	4	110	−0.4	6/6
	2	140	−0.3	6/6
	1	125	−0.3	6/6
13	16	167	−1.3	6/6
	8	156	−0.8	6/6
	4	139	−0.3	6/6
	2	117	−0.6	6/6
14	16	100	−0.7	6/6
	8	139	+0.2	6/6
	4	100	0	6/6
	2	111	+1.0	6/6
15	16	140	−0.9	6/6
	8	155	−0.4	6/6
	4	130	−0.2	6/6
	2	115	−0.3	6/6
16	4	156	−1.3	6/6
	2	156	+0.7	6/6
	1	144	+1.3	6/6
	0.5	133	+1.3	6/6
	0.25	106	+1.3	6/6
17	16	130	+0.1	6/6
	8	150	−0.1	6/6
	4	120	−1.2	6/6
	2	110	+0.3	6/6
18	16	100	+2.4	6/6
	8	100	+3.5	6/6
	4	111	+2.0	5/5
	2	100	+2.8	6/6

[a] Ascites tumor cells (10^6) were implanted ip into CDF female mice. The drug was administered ip on days 1, 5, and 9. It is considered toxic if < 4/6 mice are alive on day 5. MST = median survival time. % T/C = (MST treated/MST control) × 100. Active compounds must have % T/C ≥ 125. [b] Results are an average of eight experiments.

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(8) K. G. R. Pachler, R. R. Arndt, and W. H. Baarschers, *Tetrahedron*, **21**, 2159 (1965).

in terms of this assay. The methoxime derivative 8 of the 2′-carbonyl group had a broader therapeutic range and greater potency than olivomycin A and it was equally efficacious. The methyl imine 6 and the 8-*O*-methyl ether 16 were equal to olivomycin A in potency and efficacy. Most of the other analogues were slightly less potent or effective.

At this time only limited conclusions can be drawn about structure–activity relationships among the analogues. It appears that the introduction of electron-withdrawing substituents at position 7 decreases activity. Since the 7-methyl group, as in chromomycin A_3 (3), increases potency, the preparation of analogues with electron-releasing groups should be undertaken. Small modifications in the side chain at position 2′ are compatible with antitumor activity, except that the semicarbazone 10 and the cyclohexylidene analogue 14 are inactive. Possibly the size of these substituents interferes with bonding to DNA. Although no quantitative determinations of lipophilicity have been made, it would seem that small lipophilic substituents, such as methoxime (e.g., 8) and methyl imine (e.g., 6), are especially beneficial to activity.

One surprising result in Table I is that compounds 15 and 16, which have lost potential sites for chelation, are among the more active analogues. It has been reported that olivomycins require chelation with magnesium or other divalent metal ions in order to bind with DNA.[7,9] Furthermore, it was shown that DNA binding parallels antitumor activity.[4] This result might indicate that alternative sites of chelation are important. Thus, 16 could chelate at positions 1 and 9, whereas both compounds could chelate in the side chain. Another possibility is that the methyl ether functionality of 15 and 16 makes complexation unnecessary. The need for complexation has been attributed to repulsion between negatively charged DNA and the anion formed by ionization of a phenolic group of olivomycin, which occurs partially at physiological pH.[7]

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. Infrared spectra were determined as KBr pellets on a Beckman IR-33 spectrophotometer. Ultraviolet absorption spectra were determined on a Cary 15 spectrophotometer. Nuclear magnetic resonance spectra were recorded on a Varian EM-360L spectrometer using tetramethylsilane as the internal standard. Optical rotations were taken on a Perkin-Elmer 241MC automatic polarimeter under the condition indicated. Elemental analyses were performed by Chemalytics, Inc., Tempe, Ariz. Analytical results were within ±0.4% of theoretical values.

2′-Dihydroolivomycin A (4). An ice-cooled solution of 1 (60 mg, 0.05 mmol) in 5 mL of methanol was treated gradually with sodium borohydride (16 mg, 0.42 mmol). After 45 min, dilute HCl was added and the mixture was poured into water and extracted two times with ethyl acetate. This extract was washed with water and brine and dried. Concentration and addition of petroleum ether gave 52 mg (87%) of 4 as the mixture of isomers, a monohydrate with mp 156–160 °C dec: $[\alpha]^{26}_{546}$ −45.00 (*c* 0.1, $CHCl_3$); IR (KBr) showed ester carbonyls at 1740 and 1720 cm^{-1} and the 1-carbonyl at 1660 cm^{-1} (strongly H bonded) but no 2′-carbonyl at 1700 cm^{-1}; UV (CH_3OH) λ_{max} 413 nm (ϵ 3.83), 335 (sh), 316 (3.77), 278 (4.47), 238 (4.40); NMR ($CDCl_3$) showed a shift in the 1′-OCH_3 from δ 3.05 to 3.5. Anal. ($C_{58}H_{86}O_{26}\cdot H_2O$) C, H.

2′-(Methylimino)olivomycin A (6). A solution of 1 (60 mg, 0.05 mmol) in 5 mL of dry methanol was treated with methylamine hydrochloride (10 mg, 0.15 mmol) and two drops of pyridine. The mixture was stirred at 25 °C for 24 h and concentrated to dryness. The residue was extracted with chloroform, washed with water, and dried over sodium sulfate. Concentration of the solution and addition of petroleum ether resulted in precipitation of 51 mg (83%) of 6 as yellow powder: mp 154–157 °C; $[\alpha]^{26}_{564}$ −35.1 (c 0.5, MeOH); IR (KBr) showed no 1700-cm^{-1} band for the 2′-carbonyl group; NMR ($CDCl_3$) δ 3.15 (s, CH_3N=). Anal. ($C_{59}H_{87}NO_{25}$) C, H, N.

Olivomycin A 2′-Oxime (7). To a solution of 1 (60 mg, 0.05 mmol) in 5 mL of dry methanol was added hydroxylamine hydrochloride (10 mg, 0.15 mmol) and 2 drops of pyridine. After 24 h at 25 °C the mixture was concentrated. The residue was washed with water, dried over sodium sulfate, and concentrated as petroleum ether was added. This procedure gave 45 mg (75%) of 7 as yellow powder: mp 150–152 °C; $[\alpha]^{26}_{546}$ −42.68 (c 0.4, MeOH); IR (KBr) showed no 2′-carbonyl at 1700 cm^{-1}; NMR ($CDCl_3$) δ 8.8 (6, HON=). Anal. ($C_{58}H_{85}NO_{26}$) C, H, N.

Olivomycin A 2′-Methoxime (8). A solution of 1 (60 mg, 0.05 mmol) in 5 mL of dry methanol was treated with methoxyamine hydrochloride (16 mg, 0.2 mmol) and 2 mL of pyridine. After 60 h at 25 °C, the mixture was concentrated, and the residue was extracted with dichloromethane, washed with water, and dried over sodium sulfate. Concentration of the solution and addition of petroleum ether gave 42 mg (68%) of 8 as yellow powder: mp 145–147 °C; $[\alpha]^{26}_{546}$ −28.2 (c 0.25, MeOH); IR (KBr) showed no 2′-carbonyl at 1700 cm^{-1}; NMR ($CDCl_3$) showed doubling in the area of the OCH_3 absorption at δ 3.6 with no change in the OCH_3 at δ 3.48. Anal. ($C_{59}H_{87}NO_{26}$) C, H, N.

Olivomycin A 2′-Phenylhydrazone (9). To a solution of 1 (60 mg, 0.05 mmol) in 5 mL of ethanol was added 0.01 mL (excess) of phenylhydrazine. After 45 h at 25 °C, the mixture was concentrated, and the residue was extracted with chloroform, washed twice with water, and dried over sodium sulfate. Concentration of the solution gave a brown solid that was purified by preparative TLC on silica gel (chloroform–methanol, 9:1). Crystallization from chloroform–petroleum ether gave 42 mg (66%) of 9 as the monohydrate, a yellow solid with mp 145–149 °C: $[\alpha]^{26}_{546}$ −46.16 (c 0.1, MeOH); IR (KBr) showed no 2′-carbonyl at 1700 cm^{-1}; NMR ($CDCl_3$) δ 7.1–7.4 (m, phenyl protons). Anal. ($C_{64}H_{90}N_2O_{25}\cdot H_2O$) C, H, N.

Olivomycin A 2′-Semicarbazone (10). A solution of 1 (60 mg, 0.05 mmol) in 5 mL of dry methanol was treated with semicarbazide hydrochloride (12 mg, 0.11 mmol) and 2 drops of pyridine. After 35 h at 25 °C, the mixture was concentrated, and the residue was extracted with chloroform, washed with water, and dried over sodium sulfate. Concentration gave a solid that was purified by preparative TLC on silica gel (chloroform–methanol, 8:2). This procedure gave 38 mg (61%) of 10 as the monohydrate, a yellow solid with mp 209–212 °C: $[\alpha]^{26}_{546}$ +246 (*c* 0.05, EtOH); IR (KBr) showed no 2′-carbonyl at 1700 but had an amide CO at 1680 cm $^{-1}$. Anal. ($C_{59}H_{87}N_3O_{26}\cdot H_2O$) C, H, N.

1-Deoxyolivomycin A (11). A solution of 1 (60 mg, 0.05 mmol) in 10 mL of ethanol was treated with 12 mg of platinum oxide and shaken in a Paar apparatus with hydrogen at 20 psi for 8 h. The mixture was filtered and concentrated. Trituration of the residual solid with petroleum ether gave 43 mg (73%) of 11 as yellow powder with mp 135–138 °C: $[\alpha]^{26}_{546}$ +8.8 (c 0.25, MeOH); IR (KBr) showed ester carbonyl group at 1740 and 1720 cm^{-1} and the 2′-carbonyl at 1700 cm^{-1} but not the 1-carbonyl at 1660 cm^{-1}; UV (CH_3OH) λ_{max} 415 nm (ϵ 3.77), 342 (sh), 278 (4.19), 239 (4.56); NMR ($CDCl_3$) showed a shift in the 9-OH proton from δ 15.5 to 10.91. Anal. ($C_{58}H_{86}O_{25}$) C, H.

8,9-*O*,*O*-Dimethylolivomycin A (15). A solution of 1 (60 mg, 0.05 mmol) in 5 mL of ether at 0 °C was treated with excess diazomethane in ether. After 20 h at 5 °C, the mixture was concentrated and the residue was crystallized from chloroform–ether. This procedure gave 45 mg (73%) of 15 as the hemihydrate, a light yellow solid with mp 140–142 °C dec: $[\alpha]^{26}_{546}$ −10.4 (c 0.27, MeOH); NMR ($CDCl_3$) showed four CH_3O groups present in the region δ 3.4–3.75, but the phenolic OH peaks at δ 15.5 and 9.6 were absent. Anal. ($C_{60}H_{88}O_{26}\cdot 0.5H_2O$) C, H.

1-Deoxy-2′-(methylimino)olivomycin A (12). A solution of 11 (50 mg, 0.42 mmol) in 2.5 mL of dry methanol was treated with methylamine hydrochloride (10 mg, 0.15 mmol) and 0.5 mL of dry pyridine. After 48 h at 25 °C, the mixture was concentrated and the residue was extracted with dichloromethane. This extract was washed with water, dried over sodium sulfate, and concentrated. The crude product was purified by preparative TLC on

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silica gel (chloroform–methanol, 9:1) and crystallization from chloroform–petroleum ether. This procedure gave 26 mg (51%) of **12** as the monohydrate, a yellow solid with mp 151–154 °C: $[\alpha]^{26}_{546}$ −23.5 (c 0.08, MeOH); IR (KBr) showed no carbonyl bands at 1700 or 1660 cm^{-1}; NMR ($CDCl_3$) δ 3.3 (CH_3N=). Anal. ($C_{59}H_{89}NO_{24}\cdot H_2O$) C, H, N.

1-Deoxy-2′-dihydroolivomycin A (5). An ice-cooled solution of 11 (55 mg, 0.46 mmol) in 10 mL of dry methanol was treated gradually with sodium borohydride (15 mg, excess). The mixture was stirred for 2 h, treated with dilute HCl, and concentrated to dryness. An acetone extract of the residue was passed through a small column of silica gel and reconcentrated. The resulting solid was purified by preparative TLC on silica gel (chloroform–methanol, 8:2) and recrystallization from acetone–ether. This procedure gave 28 mg (50%) of **5** as yellow solid with mp 210 °C dec: $[\alpha]^{26}_{546}$ −17.5 (c 0.64, $CHCl_3$); IR (KBr) showed no carbonyl bands at 1700 or 1660 cm^{-1}, but the ester carbonyls were present at 1735 and 1720 cm^{-1}. Anal. ($C_{58}H_{88}O_{25}$) C, H.

3′,4′-*O*-Isopropylideneolivomycin A (13). A mixture of 1 (60 mg, 0.05 mmol), 4 mL of 2,2-dimethoxypropane, and a small amount of *p*-toluenesulfonic acid was stirred at 25 °C for 35 min, treated with a few drops of water, and concentrated. The residue was extracted with chloroform. This extract was washed with dilute sodium bicarbonate solution and with water, dried over sodium sulfate, and concentrated. Purification of the crude product by preparative TLC on silica gel (chloroform–methanol, 9:1) and crystallization from chloroform–petroleum ether gave 41 mg (67%) of **13** as the trihydrate, a yellow solid with mp 178–180 °C; $[\alpha]^{26}_{546}$ −42.00 (c 0.05, $CHCl_3$); NMR ($CDCl_3$) showed two new CH_3 groups at δ 1.25 and the phenolic OH groups were still present at δ 9.6 and 15.5. Anal. ($C_{61}H_{86}O_{26}\cdot 3H_2O$) C, H.

3′,4′-*O*-Cyclohexylideneolivomycin A (14). A solution of 1 (60 mg, 0.05 mmol) in 0.5 mL of dry tetrahydrofuran was treated with 0.5 mL (excess) of 1,1-dimethoxycyclohexane and a small crystal of *p*-toluenesulfonic acid. After 16 h at 25 °C, the mixture was poured into dilute sodium bicarbonate solution and extracted with dichloromethane. This extract was washed with water, dried over sodium sulfate, and concentrated. The residual solid was purified by preparative TLC on silica gel (chloroform–methanol, 9:1), which afforded 48 mg (75%) of **14** as the monohydrate yellow solid with mp 155–158 °C: $[\alpha]^{26}_{546}$ −47.00 (c 0.1, $CHCl_3$); NMR ($CDCl_3$) showed broadening and increase in area for absorption near δ 1.35. Anal. ($C_{64}H_{92}O_{26}\cdot H_2O$) C, H.

5-Bromo- or 7-Bromoolivomycin A (17). A solution of 1 (30 mg, 0.025 mmol) in 2.5 mL of dry dichloromethane was treated with pyridine perbromide (7 mg, 0.029 mmol). After 20 h at 25 °C, the mixture was diluted with water and dilute sodium bicarbonate solution and dried over sodium sulfate. Concentration and addition of petroleum ether gave 22 mg (68%) of **17** as yellow solid with mp 149–153 °C: $[\alpha]^{26}_{546}$ −17.08 (c 0.07, $CHCl_3$); UV (CH_3OH) λ_{max} 415 nm (ε 4.05), 340 (sh), 279 (5.60), 230 (4.36); NMR ($CDCl_3$) showed in the aromatic region 1 proton at δ 6.5 (decrease from 2) and 1 proton at δ 6.75. Anal. ($C_{58}H_{83}BrO_{26}$) C, H, Br. A small amount of a second product was obtained, but it was too unstable for purification and analysis.

5-Nitro- or 7-Nitroolivomycin A (18). A solution of 1 (60 mg, 0.05 mmol) in 5 mL of dry methanol was treated with 2 mL of phosphate buffer (pH 8.2), cooled in an ice bath, and treated with tetranitromethane (15 mg, 0.076 mmol). After 16 h at 5 °C, the mixture was concentrated and the residue was extracted with dichloromethane. This extract was washed with water, dried over sodium sulfate, and concentrated with the addition of petroleum ether. The dark product was purified by preparative TLC on silica gel (chloroform–methanol, 8:2) and crystallization from dichloromethane–ether. This procedure gave 30 mg (48%) of **18** as tan solid with mp 200–203 °C: $[\alpha]^{26}_{546}$ −33.71 (c 0.18, MeOH); IR (KBr) 1530, 1350 cm^{-1} (NO_2); UV (CH_3OH) λ_{max} 402 nm (ε 3.80), 273 (4.32), 223 (sh); NMR ($CDCl_3$) showed 1 proton at δ 6.5 (decrease from 2) and 1 proton at δ 6.8 in the aromatic region. Anal. ($C_{58}H_{83}NO_{28}$) C, H, N.

8-*O*-Methylolivomycin A (16). A mixture of 1 (60 mg, 0.05 mmol), *N,N*-dimethylformamide dimethyl acetal (2 mL), and a small amount of *p*-toluenesulfonic acid was stirred at 25 °C for 36 h, poured into dilute sodium bicarbonate solution, and extracted two times with dichloromethane. The extract was washed with brine, dried over sodium sulfate, and concentrated. Purification of the solid residue by preparative layer chromatography on silica gel (chloroform–methanol, 9:1) gave 38 mg (62%) of **16** as yellow powder with mp 183–186 °C: $[\alpha]^{26}_{546}$ −21.1 (c 0.25, MeOH); IR (KBr) 1740, 1720, 1710, 1620 cm^{-1}; UV (CH_3OH) λ_{max} 410 nm (ε 3.71), 3.15 (sh), 278 (4.31), 224 (3.94); NMR ($CDCl_3$) showed one OCH_3 at δ 3.5 and two OCH_3 at δ 3.6. The phenolic 9-OH was present at δ 15.5, whereas the 8-OH at δ 9.6 was absent. Anal. ($C_{59}H_{86}O_{26}\cdot 1.5H_2O$) C, H.

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2,4-Diamino-5-benzylpyrimidines and Analogues as Antibacterial Agents. 2. C-Alkylation of Pyrimidines with Mannich Bases and Application to the Synthesis of Trimethoprim and Analogues[1]

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A new route to 5-(*p*-hydroxybenzyl)pyrimidines has been developed which utilizes phenolic Mannich bases plus pyrimidines containing at least two activating groups. The products can be alkylated on the phenolic oxygen or on the pyrimidine N-1 atom, depending on conditions. This method has been used to prepare trimethoprim, a broad-spectrum antibacterial agent, starting from 2,4-diaminopyrimidine and 2,6-dimethoxyphenol.

The importance of trimethoprim [**13**, 2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine][2] as a broad-spectrum antibacterial agent[3-6] has dictated the need for new and improved synthetic routes. The original synthesis[2] was lengthy. Stenbuck and co-workers[7] reported a two-step

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(2) For Paper 1 of this series, see: B. Roth, E. A. Falco, G. H. Hitchings, and S. R. M. Bushby, *J. Med. Pharm. Chem.*, **5**, 1103 (1962).

(3) S. R. M. Bushby and G. H. Hitchings, *Br. J. Pharmacol. Chemother.*, **33**, 72 (1968).

(4) J. J. Burchall and G. H. Hitchings, *Mol. Pharmacol.*, 1, 126 (1965).

(5) "Evaluations on New Drugs: Trimethoprim-Sulfamethoxazole", *Drugs*, 1, 7 (1971), and references therein.

(6) "Symposium on Trimethoprim-Sulfamethoxazole", *J. Infect. Dis.*, **128** (supplement), (1973).

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