78. TETRAMETHOPRIM and PENTAMETHOPRIM: Synthesis, Antibacterial Properties and X-Ray Structures

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Dedicated to Professor *Rezsõ Bognár* on the occasion of his 70th birthday

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Summary

TETRAMETHOPRIM (**9**) and PENTAMETHOPRIM (**10**), both prepared from the corresponding benzaldehydes **3** and **6** by conventional procedures, did not exhibit noteworthy antibacterial activity *in vitro*. A single crystal X-ray analysis of **9** and **10** provided evidence that the out-of-plane methoxy groups in the two compounds created a completely different topographical situation to that present in TRIMETHOPRIM, and less ideal for the binding to bacterial dihydrofolate reductase.

The data accumulated from the testing of many analogs of TRIMETHOPRIM (TMP, **1**) clearly demonstrate that the aromatic substitution in the benzene portion of the molecule is critical for antibacterial activity [1–4]. Diaveridine (**2**), lacking one of the three vicinal methoxy groups of **1** has still noteworthy, but much weaker antibacterial activity [5]. Recent results, obtained from X-ray crystallographic data of TMP in complex with dihydrofolate reductase (DHFR) from *E. Coli* [6], and from a ^{1}H-NMR. analysis of TMP in complex with other DHFR-species [7], were used to explain the superior activity of TMP over analogs on the basis of a biochemical rationale. It was concluded that the out-of-plane methyl group at O–C(4′) of TMP forces the methyl groups at O–C(3′) and O–C(5′) into planar arrangements [8] [9], thus providing an ideal set-up for the interaction of TMP with bacterial DHFR [2]. Very recently a combination of multiple regression analysis, X-ray crystallography and computer graphics, together with the biochemistry of DHFR, provided data which led to similar conclusions [10].

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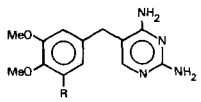
We now report that TETRAMETHOPRIM (**9**) and PENTAMETHOPRIM (**10**), two higher methoxy substituted analogs of TMP, and prepared from the corresponding benzaldehydes **3** and **6** by conventional procedures [11], did not exhibit noteworthy bacterial properties *in vitro*, nor did they mimic the broad antibacterial spectrum noted with TMP (see *Table 1*).

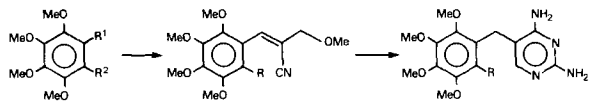
Syntheses *(Scheme).* – The known pentamethoxybenzaldehyde (**6**) [12] was prepared here from the known tetramethoxybenzaldehyde (**3**) [13] [14] by the following sequence of reactions. *Baeyer-Villiger* oxidation of **3** with *m*-chloroperbenzoic acid afforded, after alkaline hydrolysis of the intermediate formate ester, the phenol **4** which was *O*-methylated with dimethyl sulfate to the oily pentamethoxybenzene (**5**) [15]. Formylation of **5** by a *Vilsmeier-Haack* reaction afforded the oily aldehyde **6**, characterized as its 2,4-dinitrophenylhydrazone and pentamethoxybenzoic acid, obtained by oxidation of **6** with $KMnO_4$ [16].

The conversion of the benzaldehydes **3** and **6** into the *β*-methoxypropionitriles **7** and **8** and the 2,4-diaminopyrimidines **9** and **10** respectively, was accomplished with the *Stenbuck* procedure [11]. The crystalline diaminopyrimidines **9** and **10** showed the expected spectral properties, and the lack of H–C(6′) in the ^{1}H-NMR. spectrum in **10**.

Scheme



1 R = OMe (TRIMETHOPRIM)
2 R = H (Diaveridine)



3 R^1 = CHO, R^2 = H
4 R^1 = H, R^2 = OH
5 R^1 = H, R^2 = OMe
6 R^1 = CHO, R^2 = OMe

7 R = H
8 R = OMe

9 R = H
10 R = OMe

Bacteriological screening. – The minimal concentrations required for the inhibition of bacterial growth *in vitro* were measured in an agar-dilution test. The microorganisms were washed prior to the inoculation with *PBA*, diluted, and 5% horse blood added to the *Mueller-Hinton* agar [17]. The marked decrease in antibacterial activity observed *in vitro*, by going from TRIMETHOPRIM (**1**) to diaveridine (**2**), TETRAMETHOPRIM (**9**) and PENTAMETHOPRIM (**10**), was manifested by 16 different organisms. TRIMETHOPRIM (**1**) is about ten times more potent against all organisms than diaveridine (**2**), whereas TETRAMETHOPRIM (**9**) showed noteworthy activity against only two organisms and PENTAMETHOPRIM (**10**) was practically inactive against all organisms *(Table 1)*. It was suggested that the impact of the polymethoxy

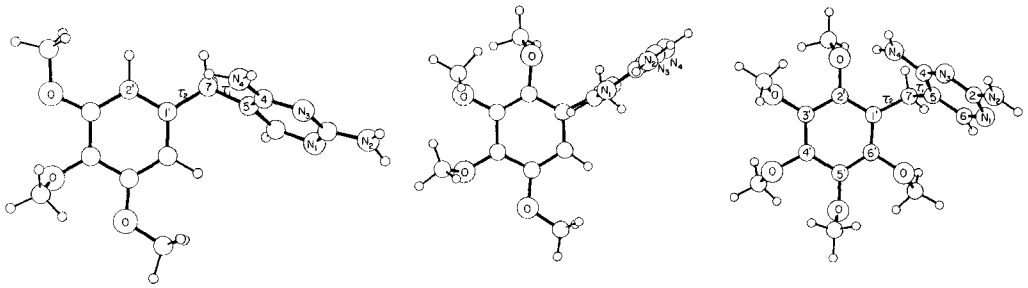
Table 1. *Antimicrobial spectra of 2,4-diaminopyrimidines*

Microorganism	Compounds			
	TRIMETHOPRIM (1)	TETRAMETHOPRIM (9)	PENTAMETHOPRIM (10)	Diaveridine (2)
Str. pyog. 308	0.025	0.781	12.500	0.391
Str. pyog. 77	0.098	1.563	25	0.781
Str. faec. MD8b	0.049	6.250	25	0.391
Staph. SG 511	3.125	25	> 100	12.500
Staph. 285	0.781	12.500	> 100	6.250
Staph. 503	1.563	12.500	> 100	6.250
E. coli 055	< 0.002	12.500	> 100	0.098
E. coli DCO	0.049	50	> 100	0.391
E. coli DC2	0.781	12.250	> 100	6.250
E. coli TEM	0.098	50	> 100	0.391
E. coli 1507E	0.195	100	> 100	0.781
Ps. aerug. 9027	> 100	> 100	> 100	> 100
Ps. aerug. 1592E	> 100	> 100	> 100	> 100
Ps. aerug. 1771	> 100	> 100	> 100	> 100
Ps. aerug. 1771m	> 100	> 100	> 100	> 100
Salm. typhimurium	< 0.002	3.125	50	0.049
Kl. aerog. 1082E	0.049	25	> 100	0.781
Kl. aerog. 1522E	0.195	> 100	> 100	1.563
Ent. cloacae P99	0.781	50	> 100	3.125
Ent. cloacae 1321E	0.781	> 100	> 100	6.250

substitution in **9** and **10** on molecular topography and conformation of the molecule, be studied in the solid state with single crystal X-ray crystallography.

Crystal structure analyses. – The conformations of TETRAMETHOPRIM (**9**) and PENTAMETHOPRIM (**10**) as established in the crystalline state are shown in the *Figure*. TETRAMETHOPRIM (**9**) crystallizes in space group $P2_1/n$ with cell parameters $a = 12.321(4)$ Å, $b = 11.842(4)$ Å, $c = 12.563(4)$ Å, $\beta = 118.9(1)°$, $Z = 4$ and refined to an agreement factor $R_w = 6.9\%$ for 660 independent intensity data measured > 0. PENTAMETHOPRIM (**10**) crystallizes in space group $P\bar{1}$ with cell parameters $a = 6.045(8)$ Å, $b = 8.327(12)$ Å, $c = 18.579(21)$ Å, $\alpha = 82.7(1)°$, $\beta = 83.5(1)°$, $\gamma = 89.0(1)°$, $Z = 2$ and refined to an agreement factor $R_w = 10.2\%$ for 1139 observed reflections. Since PENTAMETHOPRIM (**10**) crystallized with difficulty, the crystal used for the diffraction analysis was an extremely thin lath of cross-section 0.02 mm $\times$ 0.06 mm.

Compared to the conformation of the biologically active TRIMETHOPRIM [9] (**1**), TETRAMETHOPRIM (**9**) and PENTAMETHOPRIM (**10**) differ from it in several conformational aspects *(Figure)*. First, with respect to the methoxy groups, in **1** $CH_3O-C(3')$ and $CH_3O-C(5')$ are nearly in the plane of the phenyl ring and the CH_3 group of $CH_3O-C(4')$ is directed upward. In **9**, only $CH_3O-C(5')$ is coplanar with the phenyl ring while the methyl moieties on C(2'), C(3') and C(4') are rotated down, up and up, respectively, with respect to the plane of the phenyl ring. In **10**, the methyl groups of the five methoxy groups are disposed regularly down, up, down,



1 **9** **10**

Figure. *Comparison of the conformations of* TRIMETHORPIM (**1**) [9], TETRAMETHOPRIM (**9**) *and* PENTAMETHOPRIM (**10**) *in the crystalline state.* The diagrams were drawn by computer using experimentally determined coordinates from X-ray diffraction analysis.

up and down. The values of the torsional angles about the C_{ring}, O-bonds are listed in *Table 2.* The second difference is the orientation of the pyrimidine ring with respect to the substituted phenyl ring. Rotations are possible about the C(5),C(7)-bond and the C(7),C(1')-bond and the values for the torsional angles are listed in *Table 2.* The orientation of the pyrimidine ring is somewhat similar in the tri- and penta-analogs, but is quite different in the tetra-analog where, N(4) is extended away from the phenyl ring while in the tri- and penta-analogs N(4) is directed toward the phenyl ring *(Figure).* The orientation of the pyrimidine ring appears to be correlated with the H-bond formation between adjacent molecules in the crystal. In the tri- and penta-compounds there are two pairs of parallel intermolecular hydrogen bonds, between N(1)...NH(2) and N(3)...HN(4) and those related by inversion centers, while in the tetra-compound there is only one pair of H-bonds, between N(1)...HN(2), while N(3) and N(4) do not participate in any H-bonding.

Conclusion. – Examination of space-filling models shows that rotation around the C(1'),C(7)- or the C(7),C(5)-axis is possible in all three analogs and the

Table 2. *Torsional angles*[a]) (deg.)

Rotation around:	TRIMETHOPRIM [9] (**1**)	TETRAMETHOPRIM (**9**)	PENTAMETHOPRIM (**10**)
C(7)–C(1')–C(2')–OCH_3		+ 108	+ 108
CH_3O–C(2')–C(3')–OCH_3	– 5	– 66	– 84
CH_3O–C(3')–C(4')–OCH_3	– 101	– 66	+ 102
CH_3O–C(4')–C(5')–OCH_3	– 172	– 178	– 85
CH_3O–C(5')–C(6')–OCH_3			+ 76
$\tau_1 \equiv$ C(4)–C(5)–C(7)–C(1')	– 89	– 171	– 71
$\tau_2 \equiv$ C(5)–C(7)–C(1')–C(2')	+ 153	+ 85	+ 107

[a]) Each of these compounds crystallizes in a centrosymmetric space group and represent one of an enantiomeric pair. The molecules of the other chirality will have torsion angles with the same absolute value, but with all signs reversed.

orientations of the heterocyclic moieties in **1**, **9** and **10** can therefore be matched, making them less likely to be the reason for the biological differences. This seems more likely to originate from the different topography of the methoxy-substituted benzene rings, with one methyl group sticking out of the plane in **1**, three in **9**, and five in **10**. Molecule **1**, therefore, is less bulky than either **9** or **10**, but more important, offers in a cooperative effort the three vicinal aromatic O-atoms for polar attraction, a situation which does not exist in either **9** or **10** where the O-atoms are shielded from exposure by neighbouring methyl groups. The exposure of the 3 neighboring O-atoms in aromatic trimethoxy-substituted compounds might indeed play an important role, as recognized in the acid-catalyzed ether cleavage of 3,4,5-trimethoxy-substituted compounds, affording rather selectively the 4-hydroxy-3,5-dimethoxy-substituted congeners [18–22].

Experimental Part

The melting points (m.p.) were taken on a *Fisher-Johns* apparatus and are uncorrected. IR. spectra were obtained on a *Beckman* 4230 instrument (cm^{-1}). ^{1}H-NMR. spectra were determined in $CDCl_3$ by a *Varian HR-220* spectrometer relative to internal TMS ($\delta = 0$ ppm). CI./MS. spectra were obtained by using a *Finnigan 1015 D* spectrometer, and EI./MS. spectra were recorded with a *Hitachi Perkin-Elmer RMU-6E* spectrometer (70 eV). Thin-layer chromatography plates were purchased from *Analtech, Inc.*, and silica gel 60 for column chromatography (230–400 mesh) was from *EM Laboratories*.

Preparation of 2,3,4,5,6-pentamethoxybenzaldehyde (**6**). A mixture of **3** (2.26 g, 10 mmol), *m*-chloroperbenzoic acid (80%, 2.15 g, 10 mmol) in CH_2Cl_2 (20 mL) was stirred at 25° for 2 h. The solution was washed with aq. $NaHCO_3$- and aq. $NaHSO_3$-solutions, dried ($MgSO_4$) and evaporated to give *2,3,4,5-tetramethoxyphenylformate*. IR. (film): 1760 and 1740. – NMR.: 8.12 (*s*, 1 H, OCHO); 6.30 (*s*, 1 H, arom. H); 3.94 and 3.86 (each *s*, each 3 H, 2 CH_3O); 3.76 (*s*, 6 H, 2 CH_3O).

A mixture of the formate (2.4 g), CH_3ONa (0.54 g, 10 mmol), and CH_3OH (20 ml) was stirred at 25° for 0.5 h, then diluted with H_2O, acidified with AcOH, and extracted with CH_2Cl_2. The organic layer was dried ($MgSO_4$) and evaporated to leave *2,3,4,5-tetramethoxyphenol* (**4**). – NMR.: 6.22 (*s*, 1 H, arom. H); 5.60 (br. *s*, 1 H, OH, exchanged with D_2O); 3.90 and 3.84 (each *s*, each 3 H, 2 CH_3O); 3.76 (*s*, 6 H, 2 CH_3O). A mixture of the phenol **4** (2.1 g), K_2CO_3 (1.38 g, 10 mmol), $(CH_3)_2SO_4$ (1.26 g, 10 mmol), and acetone (50 ml) was refluxed for 15 h. The mixture was diluted with H_2O, extracted with Et_2O. The organic layer was dried ($MgSO_4$) and evaporated to yield *1,2,3,4,5-pentamethoxybenzene* (**5**). – NMR.: 6.22 (*s*, 1 H, ArH); 3.92 (*s*, 6 H, 2 CH_3O); 3.80 (*s*, 9 H, 3 CH_3O). Pentamethoxybenzene (**5**) (2.05 g) was added to a mixture of *N*-methylformanilide (2.03 g, 15 mmol) and phosphoroxychloride (2.30 g, 15 mmol). The mixture was stirred at 25° for 15 h and poured into ice, extracted with Et_2O. The organic layer was dried ($MgSO_4$) and evaporated to leave **6** (2.08 g, 81% from **3**); b.p. 150°/0.5 Torr. – IR. (film): 1695. – NMR.: 10.25 (*s*, 1 H, CHO); 4.00 (*s*, 3 H, CH_3O); 3.91 and 3.86 (each *s*, each 6 H, 4 CH_3O). – CI./MS. (NH_3): 257 $(M+1)^+$.

$C_{18}H_{20}N_4O_9$ (436.23) Calc. C 49.54 H 4.62 N 12.84% Found C 49.49 H 4.63 N 12.61%

2,4-Dinitrophenylhydrazone of **6**: m.p. 171–172° (EtOH). Oxidation of **6** with $KMnO_4$ in water afforded the known pentamethoxybenzoic acid; m.p. 94–95° ([12]: 95.5°).

Preparation of β-methoxy-α-(2,3,4,5-tetramethoxybenzylidene)propionitrile (**7**). A mixture of **3** (6.0 g, 26.5 mmol), 3-methoxypropionitrile (2.5 g, 29.4 mmol), CH_3ONa (0.7 g, 13 mmol), and CH_3OH (100 ml) was refluxed for 4 h, then diluted with H_2O and extracted with CH_2Cl_2. The organic layer was dried ($MgSO_4$) and evaporated to leave an oil, which was chromatographed on silica gel with hexane/Et_2O 1:1 to give **7** (5.6 g, 72%); b.p. 105–110°/0.5 Torr. – IR. (film): 2220 and 1595. – NMR.: 7.36 and 7.30 (each *s*, each 1 H, arom. and vinyl H); 4.11 (*s*, 2 H, CH_2OCH_3); 3.89, 3.86, 3.84 and 3.77 (each *s*, each 3 H, 4 CH_3O); 3.39 (*s*, 3 H, CH_2OCH_3). – CI./MS. (NH_3): 311 $([M+18]^+)$, 294 $([M+1]^+)$ and 293 (M^+). – EI./MS.: 293 (M^+).

β-Methoxy-α-(2,3,4,5,6-pentamethoxybenzylidene)propionitrile (**8**). A mixture of **4** (1.28 g, 5 mmol), 3-methoxypropionitrile (0.425 g, 5 mmol), CH_3ONa (0.135 g, 2.5 mmol), and CH_3OH (10 ml) was refluxed for 15 h, then worked up as above. Chromatography on silica gel with hexane/Et_2O 1:1 afforded **8** (1.22 g, 76%); b.p. 150°/1 Torr. – IR. (film): 2220 and 1580. – NMR.: 7.09 (*s*, 1 H, vinyl. H); 4.27 (*s*, 2 H, CH_2OCH_3); 3.98 (*s*, 3 H, CH_3O); 3.86 and 3.82 (each *s*, each 6 H, 4 CH_3O); 3.45 (*s*, 3 H, CH_2OCH_3). – CI./MS. (NH_3): 341 ($[M+18]^+$) and 323 (M^+). – EI./MS.: 323 (M^+).

Preparation of 2,4-diamino-5-(2,3,4,5-tetramethoxybenzyl)pyrimidine (**9**). A mixture of **7** (1.47 g, 5 mmol), guanidine · HCl (1.43 g, 15 mmol), CH_3ONa (0.81 g, 15 mmol), and CH_3OH (20 ml) was refluxed for 15 h. The mixture was diluted with H_2O, extracted with CH_2Cl_2, and the organic layer was dried ($MgSO_4$) and evaporated. The residue was chromatographed on silica gel with CH_2Cl_2/MeOH/NH_4OH 90:9:1 to give **9** (610 mg, 38%); m.p. 156–157° (CH_2Cl_2/hexane). – IR. (KBr): 3440, 3360, 1645, 1615, 1600, 1560. – NMR.: 7.66 (*s*, 1 H, pyrimidyl-H); 6.18 (*s*, 1 H, phenyl-H); 5.23 and 4.73 (each br. *s*, each 2 H, 2 NH_2, disappeared with D_2O exchange), 3.82, 3.80, 3.77, and 3.68 (each *s*, each 3 H, 4 CH_3O); 3.50 (*s*, 2 H, ArCH_2Ar). – CI./MS. (NH_3): 321 ($[M+1]^+$). – EI./MS.: 320 (M^+).

$C_{15}H_{20}N_4O_4$ (320.28) Calc. C 56.24 H 6.29 N 17.49% Found C 55.98 H 6.21 N 17.26%

Preparation of 2,4-diamino-5-(2,3,4,5,6-pentamethoxybenzyl)pyrimidine (**10**). A mixture of **8** (626 mg, 2 mmol), guanidine · HCl (860 mg, 9 mmol), CH_3ONa (486 mg, 9 mmol), and CH_3OH (10 ml) was refluxed for 45 h. Workup as above followed by chromatography on silica gel with CH_2Cl_2/MeOH/NH_4OH 90:9:1 afforded the starting nitrile **8** (360 mg) and the desired pyrimidine **10** (190 mg, 27%); m.p. 216–217° (acetone). – IR. (KBr): 3480, 3420, 1660, 1630, 1600, 1570. – NMR.: 7.91 (*s*, 1 H, arom. H); 5.60 and 4.70 (each br. *s*, each 2 H, 2 NH_2); 3.76 (*s*, 15 H, 5 CH_3O); 3.51 (*s*, 2 H, ArCH_2Ar). – CI./MS.: (NH_3): 351 ($[M+1]^+$).

$C_{16}H_{22}N_4O_5$ (350.39) Calc. C 54.85 H 6.33 N 15.99% Found C 54.70 H 6.06 N 15.64%

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