Degradation of Trimethoprim

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Abstract □ Trimethoprim undergoes thermally and photochemically catalyzed hydrolysis or oxidation to give rise to at least five products. The structures of these compounds were determined by physical methods and it was found that the resonance of C-5 in the ^{13}C NMR spectrum is indicative of the substitution pattern of the resultant amino hydroxy pyrimidines.

Although trimethoprim (1) is a relatively stable drug, it is susceptible to degradation under certain conditions. During degradation in acidic and alkaline mediums at elevated temperatures or under the influence of direct sunlight, we have isolated the five degradation products 2–6. Preliminary studies have indicated that at least two of these degradation products (4 and 5) are present in commercial pharmaceutical suspensions.

Previous investigations have shown that 1 is metabolized extensively. Several metabolites were isolated from the urine of humans,[1] dogs,[1,2] rats,[1] pigs,[3,4] goats,[3,4] and cows.[3] Some of these metabolites were synthesized.[5,6] (See Table I.)

Very little is known, however, about the chemical behavior of this drug and of its possible degradation products. Mention has been made of the existence of two degradation products,[7] and recently we reported a stability-indicating high-performance liquid chromatographic analysis of 1 in pharmaceuticals.[8]

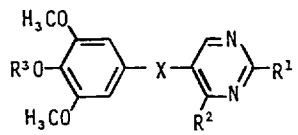
The purpose of this investigation was to determine the behavior of 1 under acidic and alkaline aqueous conditions at elevated temperatures, under the influence of direct sunlight in suspensions at different pH values, and in methanolic solution, as well as to identify the degradation products thus obtained. The influence of metal ions on the degradation was not studied.

Results and Discussion

During the degradation of trimethoprim (1) under various conditions, the formation of six products was observed. Column chromatography on silica gel afforded the five pure compounds 2–6. Insufficient material prevented the isolation of the sixth product, a substance which turns yellow in daylight on the TLC plate having a R_f value of 0.22 in the chromatographic system used (Table II). The structures of the isolated compounds were established mainly by IR, ^1H and ^{13}C NMR, and mass spectrometry. The NMR spectral and chromatographic data are summarized in Tables II, III, and IV.

Degradation product 2 was obtained as a white powder exhibiting strong absorption bands at 1580–1600 cm^{-1} in the IR spectrum attributable to a carbonyl group. The accurate mass of 304.1175 suggests a molecular formula of $C_{14}H_{16}N_4O_4$, implying the oxidation of the methylene group of 1 to a carbonyl function. The ^1H NMR spectrum (Table II) differs from that of 1 in the downfield shifts of 0.65 and 0.26 ppm, respectively, for the signals due to H-2′,6′ and H-6, as well as in the absence of the methylene resonance. The ^{13}C NMR spectrum (Table III) clearly displays the carbonyl singlet at δ 193.10 and no methylene signal. This compound probably arises via radical mechanism during the photochemical reaction.

Table I—Structures of Trimethoprim (1) and Its Degradation Products (2–7)



	R^1	R^2	R^3	X
1	NH_2	NH_2	CH_3	-CH-
2	NH_2	NH_2	CH_3	-C- (O)
3	OH	OH	CH_3	$-CH_2-$
4	OH	NH_2	CH_3	$-CH_2-$
5	NH_2	OH	CH_3	$-CH_2-$
6	NH_2	OH	H	$-CH_2-$
7	NH_2	NH_2	H	$-CH_2-$

Compound 3 crystallized as large needles with an IR spectrum very similar to that of 1. The accurate mass of 292.1050 obtained for 3 indicates a molecular formula of $C_{14}H_{16}N_2O_5$, which was confirmed by elemental analysis. The ^1H NMR spectrum (Table II) is almost identical to that of 1 with the exception of the downfield shift of 0.34 ppm in the signal of H-6 and differences in the D_2O-exchangeable resonances. In the ^{13}C NMR spectrum (Table IV), characteristic but shifted signals arising from the pyrimidine ring are observed. These data suggest a structure similar to that of 1, but with both amino groups on the heterocyclic ring having been replaced by hydroxyl functions. It was noted that in compounds having two amino substituents on the pyrimidine ring (1 and 2), the signal from C-2 appeared above 160 ppm, while in products having one or no amino groups (3–7) on this ring, the resonance of C-2 was in the region well below 160 ppm (Table IV).

From MS data it was clear that the remaining three degradation products (4–6) each had an amino group and a hydroxy group as substituents on the pyrimidine ring. Compounds 4 and 5 both analyzed for $C_{14}H_{17}N_3O_4$. The relative

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Table II—Chromatographic Data

Compound	R_f[a]	Retention time, min[b]
1	0.41	4.37
2	0.68	2.16
3	0.74	1.09
4	0.54	2.71
5	0.65	2.22
6	0.59	2.25

[a] The TLC was carried out on 0.25-mm silica gel plates (Merck 5735) with methanol:chloroform (2:1) as eluant. [b] Performed on a Zorbax TMS 7-μm column with acetonitrile:1-propanol:methanol:tetrahydrofuran: acetic acid:water (5:20:15:25:1:34) at 2 mL/min.

Table III—^{1}H NMR Chemical Shifts[a]

Compound	H-6	H-2',6'	CH_2	3',5'-OMe	4'-OMe	NH_2	OH
1	7.53	6.55	3.53	3.72	3.62	6.14 5.77	—
2	8.18	6.81	—	3.74	3.72	—	—
3	7.19	6.53	3.42	3.71	3.60	—	10.94
4	7.05	6.56	3.48	3.72	3.61	—	10.40
5	7.41	6.52	3.45	3.71	3.60	6.76[b]	11.31[b]
6	7.34	6.47	3.41	3.70	—	6.72[b]	8.03[b] 11.30[b]

[a] Chemical shifts (δ) in ppm downfield from tetramethylsilane (TMS) in Me_2SO-d_6. [b] Signals bearing the same superscript in any row may be interchanged.

Table IV—^{13}C NMR Chemical Shifts[a]

	1	2	3	4	5	6
C-2	162.20	163.84	151.34	156.58	155.36	154.77
C-4	162.20	163.42	164.36	165.53	163.91[d]	163.62
C-5	105.75	103.42	111.88	103.79	114.37	115.11
C-6	155.69	164.16	138.78	140.85	—[c]	—[c]
C-7	32.98	193.10	31.98	32.41	32.43	32.15
C-1'	135.83[b]	134.64[b]	135.95[b]	134.45[b]	136.64[b]	133.77[b]
C-2',6'	105.87	105.97	105.99	106.08	105.91	106.17
C-3',5'	152.68	152.48	152.73	152.79	152.68	147.81
C-4'	135.77[b]	139.56[b]	135.59[b]	136.50[b]	135.84[b]	130.42[b]
3',5'-(OMe)	55.80	56.02	55.87	55.87	55.76	55.97
4'-OMe	59.90	60.06	60.00	59.98	59.86	—

[a] Chemical shifts (δ) in ppm downfield from tetramethylsilane in Me_2SO-d_6. [b] Signals bearing the same superscript in any column may be interchanged. [c] Signal not observed. [d] Signal sometimes not observed.

positions of the two functional moieties on the heterocyclic ring (viz. 2-amino-4-hydroxy- versus 4-amino-2-hydroxy-pyrimidine) were determined by ^{13}C NMR spectroscopy. In these spectra of all the compounds, the signal of C-5 could unequivocally be identified as the singlet at ~δ 100–115. This resonance is indicative of the substitution pattern since it consistently appeared in two nonoverlapping regions: in the pyrimidines bearing a 4-amino group, this signal was observed in the region $\delta < 110$, whereas in the pyrimidines having a hydroxyl function in this position, this signal appeared in the region >110 ppm. This distinction seems to be more widely applicable to pyrimidines since it agrees with data from several pyrimidine nucleosides,[9,10] although this phenomenon was not mentioned previously. It is also supported by the resonances of selectively ^{13}C-enriched trimethoprim.[11]

Thus, **4**, with an accurate mass of 291.1220 corresponding to the molecular formula of $C_{14}H_{17}N_3O_4$, is 4-amino-2-hydroxy-5-(3,4,5-trimethoxybenzyl)pyrimidine on the basis of the chemical shift of C-5 at δ 103.79. This structure corresponds with that of an authentic sample synthesized by Wellcome[12] through condensation of 1-(3,4,5-trimethoxybenzyl)-2-anilino acrylonitrile with thiourea, followed by hydrolysis of the 2-mercapto group of the resulting 4-amino-2-mercapto-5-(3,4,5-trimethoxybenzyl)pyrimidine with acetic acid.

The isomeric product **5**, in which the resonance of C-5 appears at δ 114.37, is the 2-amino-4-hydroxy derivative.

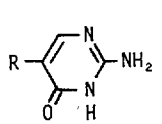
Product **6** on standing turned yellow in daylight on TLC plates and was obtained as fine yellow needles (mp 238 °C) from ethanol. From the ^{1}H and ^{13}C NMR spectra (Tables III and IV) it is evident that the 4'-methoxy group is absent, while the accurate mass of 277.1046, supporting a molecular formula of $C_{13}H_{15}N_3O_4$, demonstrates that this methoxy group is replaced by a hydroxyl function. The substitution of the pyrimidine ring is identical to that of **5**: the resonance of C-5 at δ 115.11 indicates the hydroxyl function at C-4. Compound **6** was also prepared by the method Nordholm and Dalgaard[6] used to synthesize a metabolite (**7**) of **1**. It, however, differed structurally from the product they obtained. In **6**, a hydroxy group was present in position 4 while the metabolite (**7**)[6] retained this amino function.

The structures of the degradation products illustrate the main decomposition of **1** under these conditions to be hydrolysis of the amino groups (leading to products **3**, **4**, **5**, and **6**), oxidation of the benzylic methylene function (product **2**), and hydrolysis of the *p*-methoxy group (product **6**). The latter decomposition, which only occurs in concentrated acid, can be understood in terms of the possible formation of an intermediate quinone methide which might also be responsible for the yellow coloration of this product on standing. Hydrolysis of the amino groups appears to occur independently leading to **4** and **5**, although **5** formed first (~2 h in 1 M HCl and ~4 h in 0.1 M HCl or in 1 M NaOH). Further hydrolysis of any of these two compounds gives rise to the dihydroxy product **3**.

The tautomerism of pyrimidinols has been the subject of many investigations and it is suggested that this tautomerism plays an important role in the biologic activity of these compounds.[13] By using all-electron calculations to determine the relative stability of several tautomeric forms of pyrimidines, Les and Kukawska-Tarnawska[13] recently predicted cytosine derivatives to exist in aqueous solution as amino-keto (**8**) and amino-enol (**9**) tautomers, with the latter probably the predominant species. The imino tautomers were predicted to be less stable to such an extent that they may be ruled out. This tautomerism might explain the broadening and the disappearance of C-4 and C-6 resonances in the ^{13}C NMR spectrum (Table IV) of **5**.

Experimental Section

Instruments—Melting points were determined with a Leitz Laborlux K microscope equipped with a Goerz Metrawatt thermocouple and are uncorrected. Infrared (IR) spectra were recorded on a Nicolet 5 DX Fourier transform infrared spectrometer for KBr pellets. Mass spectrometry was done on a VG 7070e or a Varian Mat 212 mass spectrometer at 70 eV. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker WM 500 Fourier transform spectrometer at 500 and 125.76 MHz, respectively, for solutions in Me_2SO-d_6, unless otherwise stated, using Me_4Si as internal standard, Where analyses are indicated by the symbols of elements only, the analytical results obtained for those elements were within ±0.4% of the theoretical values.



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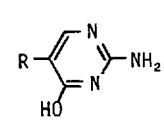

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Chromatography—The TLC was done on precoated silica gel plastic sheets (Merck 5735) with visualization accomplished by fluorescence quenching under UV light (254 nm) and R_f values refer to this technique. Methanol:chloroform (2:1) was used as the mobile phase. Column chromatography was done on open glass columns packed with silica gel (Merck 9385). The HPLC was executed[8] on a Knauer instrument using a stainless-steel column packed with Zorbax TMS 7 μm (DuPont) and deaerated acetonitrile:1-propanol:methanol:tetrahydrofuran:acetic acid:water (5:20:15:25:1:34) at a flow rate of 2 mL/min.

Materials—Trimethoprim (1), obtained from The Wellcome Foundation and from Propan South Africa, was used as received. All other chemicals were reagent grade.

Degradation of Trimethoprim—Trimethoprim (1) was refluxed in distilled water, phosphate buffer solution (pH 7), 0.1 M HCl, 1 M HCl, or in 1 M NaOH, respectively. It was also dissolved in methanol or suspended in various aqueous buffer solutions (pH 4.5 to 8.0) in 100-mL flat, clear glass bottles and exposed to direct sunlight. These reactions were monitored by TLC or HPLC.

The ketone 2 was only detected in the samples exposed to sunlight, while 3–6 were formed in at least one of the refluxed media. The products were separated by column chromatography using chloroform:ethanol:benzene (6:3:1) or ethyl acetate:ethanol:chloroform (6:3:1) as mobile phases, and purified by crystallization from methanol:chloroform (1:1) unless otherwise stated. Compound 6 was also obtained by a procedure[6] previously used to prepare a metabolite of 1.

2,4-Diamino-5-(3,4,5-trimethoxybenzoyl)pyrimidine (2)—Fine white needles, mp 190 °C [methanol:chloroform (1:1)], $\nu_{max}(cm^{-1})$: 3410, 1600, 1580, 1490, 1400, 1350, 1250, 1220, 1120, 990, 822, 800, 760; *m/z*(%): 305 (M^++1, 18), 304 (M^+, 95), 303 (59), 274 (11), 273 (59), 261 (14), 259 (13), 257 (12), 229 (15), 137 (69), 95 (100), 81 (11), 77 (10), 68 (21).

2,4-Dihydroxy-5-(3,4,5-trimethoxybenzyl)pyrimidine (3)—Large white needles, mp 206 °C [methanol:chloroform (1:1)], $\nu_{max}(cm^{-1})$: 3220, 3130, 3030, 2850, 1718, 1670, 1585, 1510, 1425, 1340, 1250, 1210, 1140, 1002, 850, 772, 695; *m/z*(%): 293 (M^++1, 26), 292 (M^+, 100), 277 (61), 261 (78), 245 (17), 234 (28), 218 (26), 206 (20), 202 (25), 191 (27), 190 (19), 175 (25), 174 (22), 148 (23), 120 (21); C, H, N.

4-Amino-2-hydroxy-5-(3,4,5-trimethoxybenzyl)pyrimidine (4)—White powder, mp 258 °C [methanol:chloroform (1:1)], $\nu_{max}(cm^{-1})$: 3230, 3090, 3040, 2960, 2920, 1650, 1510, 1470, 1430, 1330, 1240, 1130, 1002, 960, 920, 837, 810, 792, 570, 520; *m/z*(%): 292 (M^++1, 13), 291 (M^+, 81), 276 (26), 274 (21), 260 (18), 259 (23), 244 (22), 233 (16), 232 (13), 216 (20), 201 (22), 190 (12), 173 (12), 81 (23), 44 (100); C, H, N.

2-Amino-4-hydroxy-5-(3,4,5-trimethoxybenzyl)pyrimidine (5)—White powder, mp 242 °C [methanol:chloroform (1:1)], $\nu_{max}(cm^{-1})$: 3320, 3070, 2940, 2840, 1660, 1650, 1590, 1490, 1460, 1425, 1380, 1340, 1310, 1240, 1190, 1135, 1012, 980; *m/z*(%): 292 (M^++1, 16), 291 (M^+, 95), 276 (24), 261 (19), 260 (100), 244 (20), 234 (11), 218 (14), 202 (14), 201 (17), 148 (16), 124 (35), 65 (15), 43 (46), 28 (30); C, H, N.

2-Amino-4-hydroxy-5-(4-hydroxy-3,5-dimethoxybenzyl)pyrimidine (6)—Fine yellow rods, mp 238 °C (ethanol), $\nu_{max}(cm^{-1})$: 3300, 1870, 1700, 1607, 1495, 1450, 1340, 1210, 1112, 1050, 980, 815, 750; *m/z*(%): 277 (M^+, 56), 246 (31), 216 (11), 202 (8), 174 (10), 166 (9), 154 (14), 124 (100), 82 (20), 77 (11).

Conclusions

Trimethoprim (1) undergoes degradation to give rise to at least five products (2 to 6). The 4-hydroxy compound 5 is formed first, while the ketone 2 is the predominant degradation product in sunlight. The ^{13}C NMR resonance of C-5 is indicative of the substitution pattern of these amino hydroxy pyrimidines.

References

1. Schwartz, D. E.; Vetter, W.; Englert, G. *Arzneim–Forsch.* **1970**, *20*, 1867.
2. Kaplan, S. A.; Weinfield, R. E.; Cotler, S.; Abruzzo, C. W.; Alexander, K. *J. Pharm. Sci.* **1970**, *59*, 358.
3. Nielsen, P.; Rasmussen, F. *Acta Pharmacol. Toxicol.* **1975**, *36*, 123.
4. Nielsen, P.; Dalgaard, L. *Xenobiotica* **1978**, *11*, 657.
5. Rey-Bellet, G.; Reiner, R. *Helv. Chim. Acta* **1970**, *53*, 945.
6. Nordholm, L.; Dalgaard, L. *Arch. Pharm. Chem.* **1983**, *11*, 241.
7. Singletary, R. O.; Sancilio, F. D. *J. Pharm. Sci.* **1980**, *69*, 144.
8. Bergh, J. J.; Breytenbach, J. C. *J. Chromatogr.* **1987**, *387*, 528.
9. Jones, J. J.; Grant, D. M.; Winkley, M. W.; Robins, R. K. *J. Am. Chem. Soc.* **1970**, *92*, 4079.
10. Breitmaier, E.; Voelter, W. *^{13}C NMR-Spectroscopy*; Verlag Chemie: Weinheim, 1974; p 244.
11. Smal, M.; Cheung, H. T. W.; Davies, P. E. *J. Chem. Soc., Perkin Trans. 1* **1986**, 747.
12. Lines, C. B. The Wellcome Foundation, private communication.
13. Les, A.; Kukawska-Tarnawska, B. *J. Mol. Struct. (Theochem.)* **1986**, *148*, 45.

Acknowledgments

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