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Oxidation and reduction of sulfamethoxazole

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Oxidation and reduction of sulfamethoxazole (SMX) was studied under various reaction conditions. Oxidation reaction by hydrogen peroxide in glacial acetic acid afforded the corresponding nitroso **1** (SMX-NO) or nitro derivative **2** (SMX-NO_2). When the reaction was catalyzed by methylrhenium trioxide (MTO) the main product was azoxy derivative **3** (SMX-AZ). Catalytic hydrogenation of SMX with Pd/$BaSO_4$ resulted in amidine **4** (SMX-AD). The compound **4** was also prepared by hydrogenation of nitro compound **2** under the same reaction conditions. Hydrogenation of **2** in the presence of PtO_2 and triethyl phosphite (TEP) gave the hydroxylamine **5** (SMX-HA). Reduction of **2** by zinc dust gave the same product. Selective oxidation of **5** led to nitroso compound **1**.

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Keywords: sulfamethoxazole, oxidation, catalytic hydrogenation

Sulfamethoxazole (SMX) is a widely used antibacterial drug. Most serious side-effects of SMX and other sulfonamides are hypersensitivity reactions, the pathogenesis of which has been suggested to be mediated by reactive metabolites (1). N^4 oxidation of SMX to the hydroxylamine (SMX-HA), nitroso (SMX-NO) or nitro metabolites (SMX-NO_2) is thought to be an important route of bioactivation (2). It has been shown that SMX is metabolized by cythochrome P-450 in liver to its hydroxylamine metabolite. Activated monocytes and neutrophils metabolize SMX to HA and nitro derivative (3). The nonenzymatic oxidation of hydroxylamine leads to nitroso derivative. The dose-related toxicity of hydroxylamine and nitroso metabolites and the reactivity towards some biological nucleophiles has been reported (4–7).

Many methods on the selective oxidation of aromatic amines to one of the oxidative products have been reported in literature. For example, the convenient method for the direct oxidation of different aromatic amines to the corresponding nitroso compounds using a mixture of glacial acetic acid and hydrogen peroxide has been described (8). If anilines were oxidized by *tert*-butyl hydroperoxide or cumyl hydroperoxide in the presence of soluble titanium compounds the corresponding azoxy compounds were obtained (9). Methylrhenium trioxide (MTO), a relatively new homogenous catalyst, an activator of hydrogen peroxide in water and organic solvents (methanol, hexane), oxidized aniline to nitrosobenzene (10).

* Correspondence

Preparation of the oxidative derivatives of SMX have been subject of several studies. The nitro derivative SMX-NO_2 was readily prepared from 4-nitrobenzenesulfonyl chloride (4-NSC) and 3-amino-5-methylisoxazole (AMI) as starting materials (4). It was further reduced to the SMX-HA with hydrogen in the presence of a poisoned platinum catalyst (4). SMX-NO was prepared by selective oxidation of SMX-HA by ferric chloride (7) and azo derivative was obtained by the electrochemical oxidation of SMX at pyrolytic graphite electrode (11). To our knowledge, other methods on direct oxidation or reduction of SMX are not described. The aim of our research was to investigate these reactions. We report here new convenient methods for preparation of SMX oxido-reduction derivatives. These products are pro-reactive or reactive metabolites of SMX which may be implicated in its toxicity. Such compounds may be useful in the development of rapid, accurate diagnostic tests for hypersensitivity of SMX.

EXPERIMENTAL

Infrared spectra were recorded on a Paragon 500 FT-IR (Perkin Elmer, UK), ^1H NMR and ^{13}C NMR on a Varian Gemini 300 MHz (Varian, UK). The mass spectra were scanned on an AutoSpec instrument (Micromass, UK) operting at 70 eV. Catalytic hydrogenation reactions were performed at room temperature and atmospheric pressure using Pd(5%)/$BaSO_4$ (12) or PtO_2 (Fluka, Switzerland). For thin layer chromatography, silica gel sheets Kieselgel 60 F254 (Merck, Germany) were used. Solvent system was chloroform/methanol in a 9:1 ratio. For spot detection *p*-dimethylbenzaldehyde (DB) solution or iodine vapours were used. Column chromatography was performed on silica gel (0.063–0.200 mm) purchased from Kemika (Croatia). SMX, AMI, and triethyl phosphite (TEP) were kindly supplied by Pliva (Croatia). MTO, 4-NSC and DB were purchased from Aldrich Chemical Co. (Aldrich, USA).

4-Nitroso-N-*(5-methyl-3-isoxazolyl)benzenesulfonamide (SMX-NO) (1)*

Reaction a. – SMX (0.253 g, 0.001 mol) dissolved in 4 mL glacial acetic acid was oxidized by 0.28 mL 30% aqueous hydrogen peroxide (0.0023 mol). The reaction mixture was stirred for 24 h at room temperature. The precipitated product **1** was filtered off and washed with water (yield 0.190 g, 66.7 %). The compound **1** was also synthesized by oxidation of SMX-HA by ferric chloride solution following the literature procedure (7) (yield 37.5%). m.p. 180–190 °C (decomp.).

IR(KBr): ν_{max} 3099, 3000, 2913, 2852, 1617, 1529, 1465, 1405, 1350, 1296, 1261, 1179, 1140, 1109, 1093, 1036, 1008, 934, 858, 793, 723 cm^{-1}.

^1H NMR spectrum was identical to the literature data (7).

4-Nitro-N-*(5-methyl-3-isoxazolyl)benzenesulfonamide (SMX-NO_2) (2)*

Reaction b. – SMX (0.253 g, 0.001 mol) in 4 mL glacial acetic acid was oxidized by 0.8 mL 30% aqueous hydrogen peroxide (0.007 mol). The reaction mixture was stirred for 46 h at room temperature. The precipitated product **2** was filtered off (yield 0.140 g, 49.5 %),

dissolved in 5% solution of sodium hydroxide and precipitated by an addition of HCl to pH 3. Finally, the product was recrystallized from methanol/ethanol.

Reaction c. – SMX (0.253 g, 0.001 mol) in 4 mL glacial acetic acid was oxidized by 0.8 mL 30% aqueous hydrogen peroxide (0.007 mol). The reaction mixture was stirred for 2 h in the boiling water bath. The crude product **2** was isolated and purified as described in the reaction *b* (yield 0.175 g, 62%).

Reaction d. – SMX (3.795 g, 0.015 mol) in 30 mL methanol, 30 mL 30% aqueous hydrogen peroxide (0.265 mol) and 1.5 mL concentrated sulfuric acid was stirred 15 h at 40 °C. Additional amount of 15 mL hydrogen peroxide solution (0.132 mol) was added in two portions, after 7 and 11 h. The crude product **2** was isolated and purified as described in the reaction *b* (yield 4.250 g, 100%).

The compound **2** was also synthesized from 4-NSC and AMI following the literature source (4) with minor changes: the starting materials were not mixed in pyridine at 0 °C but AMI (5.88 g, 0.06 mol) was added slowly to the NSC solutions (13.30 g, 0.06 mol) in 30 mL of dry pyridine. Recrystallization from ethyl acetate/toluene (1:3) mixture yielded 56% of **2** (48% reported in ref. 4). m.p. 196–198 °C.

IR (KBr): ν_{max} 3455, 3200–2700, 1616, 1539, 1474, 1407, 1347, 1300, 1264, 1182, 1135, 1104, 1090, 1041, 1004, 940, 851 cm^{-1}.

^1H NMR (DMSO-d_6), δ (ppm): 11.89 (s, 1H, SO_2NH), 8.43 (d, 2H, ar.), 8.12 (d, 2H, ar.), 6.18 (s, 1H, =CH), 2.31 (s, 3H, CH_3).

Azoxybenzene-4,4-(N-5-methyl-3-isoxazolyl)disulfonamide (SMX-AZ) (3)

Reaction e. – To a solution of 0.507 g (0.002 mol) SMX in 8 mL methanol, 2 mL 30% aqueous hydrogen peroxide (0.018 mol) and 0.025 g (0.1 mmol) MTO was added. The reaction mixture was stirred for 1.5 h at room temperature. The precipitation of yellow product **3** started immediately. The product was filtered off (yield 0.417 g, 80.4%). From the mother liquor 15% of nitro compound **2** was isolated. m.p. > 210 °C.

IR(KBr): ν_{max} 3437, 2922, 2852, 1616, 1477, 1405, 1348, 1268, 1177, 1091, 1042, 938, 845, 746 cm^{-1}.

Elemental analysis (%):	calcd.	C 46.33	H 3.50	N 16.21	S 12.37
$C_{20}H_{18}N_6O_7S_2$ (518.52)	found	46.17	3.34	15.89	12.32

^1H NMR (DMSO-d_6), δ (ppm): 11.75 (s, 2H, SO_2NH), 8.47 (d, 2H, ar.), 8.13 (t, 4H, ar.), 8.03 (d, 2H, ar.), 6.18 (s, 2H, =CH), 2.32 (s, 6H, CH_3).

MS m/z (relative intensity, %): 517 $[M-1]^+$ (85), 505 (16), 485 (12), 472 (65), 459 (100), 439 (11), 352 (31), 341 (13), 335 (9), 319 (62.5).

MS m/z (relative intensity, %): 519 $[M+1]^+$ (34.5), 460 (100), 443 (23), 428 (6).

N-*(4-Aminobenzenesulfonyl)-3-oxo-butyramidine (SMX-AD) (4)*

Reaction f. – A mixture of 0.253 g (0.001 mol) SMX, 5 mL dry dioxane and 0.253 g Pd(5%)/$BaSO_4$ was hydrogenated for 5 h. The catalyst was removed by filtration and the solvent evaporated under the reduced pressure, yielding the crude product **4**. After recrystallization from ethanol, 0.209 g (82%) of pure **4** was obtained.

Reaction g. – A mixture of 0.570 g (0.002 mol) SMX-NO_2 in 20 mL dry ethyl acetate and 0.540 g Pd(5%)/$BaSO_4$ was hydrogenated for 5 h. The catalyst was removed by filtration and the solvent evaporated under the reduced pressure, yielding 0.47 g (92%) of the crude product **4**.

Reaction h. – An analogous reaction as the reaction *g*, but the reaction was run in dry dioxane. m.p. 93–96 °C.

IR(KBr): ν_{max} 3377, 3334, 3243, 2917, 1711, 1637, 1593, 1533, 1502, 1418, 1371, 1335, 1307, 1262, 1168, 1143, 1084, 937, 835, 792, 727, 707 cm^{-1}.

Elemental analysis (%):	calcd.	C	47.04	H	5.13	N 16.45	S 12.56
$C_{10}H_{13}N_3O_3S$ (255.26)	found		47.04		5.29	16.36	12.39

^1H NMR (DMSO-d_6), δ (ppm): 13.95 (s, 1H, SO_2NH), 8.34 (s, 1H, C=NH), 7.43 (d, 2H, ar.), 6.57 (d, 2H, ar.), 5.85 (s, 2H, NH_2), 3.45 (s, 2H, CH_2), 2.07 (s, 3H, CH_3).

^{13}C NMR (DMSO-d_6), δ (ppm): 201.93 (CO), 162.71 (ar.), 152.46 (ar.), 128.07 (2C ar.), 112.58 (2C, ar.), 90.92 (C=N), 50.03, (CH_2), 29.71 (CH_3).

MS m/z (relative intensity, %): 254 $[M-1]^+$ (67), 243 (9), 212 (8), 205 (36), 196 (8), 183 (100), 165 (6), 151 (6), 113 (10), 91 (47), 71 (9), 59 (8).

MS m/z (relative intensity, %): 256 $[M+1]^+$ (62), 242 (8), 226 (4), 214 (4.5), 197 (4.5), 167 (6), 154 (100), 149 (12), 136 (71.5), 124 (10), 120 (13.5), 115 (5), 107 (24), 95 (10), 89 (20), 81 (10), 77 (20), 69 (16.5), 65 (9.5), 55 (17).

*4-Hydoxylamino-*N*-(5-methyl-3-isoxazolyl)benzenesulfonamide (SMX-HA) (5)*

Reaction i. – The compound was synthesized following the procedure (3). m.p. 147–149 °C; m.p. 149 °C (4).

IR(KBr): ν_{max} 3476, 3315, 3246, 3160–2700, 2362, 1613, 1594, 1488, 1472, 1399, 1368, 1338, 1267, 1182, 1162, 1140, 1092, 1033, 1007, 938, 882, 835, 785, 761 cm^{-1}.

^1H NMR spectrum was identical to that reported in ref. 7.

MS m/z (relative intensity, %): 270 $[M+1]^+$ (100), 254 (5.5), 167 (6), 154 (45.5), 149 (23), 136 (43), 123 (14), 115 (5), 11 (10), 107 (23.5), 99 (22), 95 (29), 91 (22), 85 (14), 81 (33), 77 (18), 73 (10), 69 (50), 55 (60).

RESULTS AND DISCUSSION

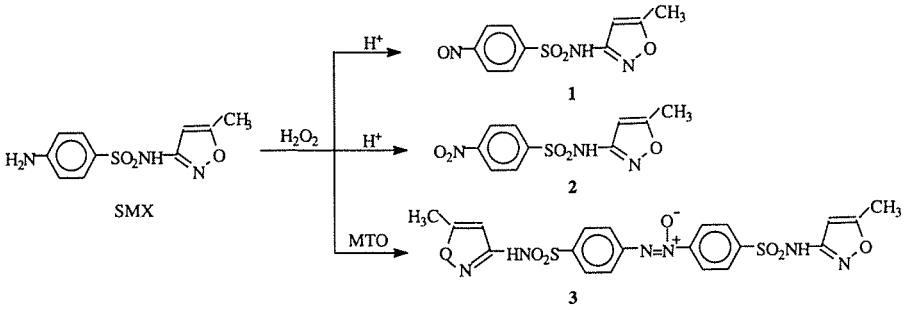
In our investigation the oxidation of SMX by hydrogen peroxide has been studied. The reactions were carried out with 30% aqueous hydrogen peroxide in glacial acetic acid (reactions *a–c*) or in methanol with catalytic amounts of sulfuric acid (reaction *d*) or MTO (reaction *e*). By varying the SMX/H_2O_2 molar ratio, catalyst, solvent and reaction time different products were obtained. If two moles of hydrogen peroxide were used to one mole of SMX the selective oxidation to SMX-NO was achieved. With the higher molar excess of H_2O_2, at an elevated temperature or by extending the reaction time, the oxidation turned to the nitro compound **2** as a main product. MTO proved to be an excel-

lent catalyst for the selective oxidation of SMX to **3** (SMX-AZ). Oxidation proceeded through dimerization to give the azoxy product **3**. The presumed intermediate, nitroso derivative was not detected during the course of reaction and in the final product. This result suggested that the highly reactive nitroso might have been converted into azoxy either by the condensation of the primary hydroxylamine intermediate with nitroso or by the condensation of nitroso with unreacted SMX, which led to the formation of azo derivative, which was further oxidized to azoxy compound.

The results of the SMX oxidation are summarized in Table I and the reactions are shown in Scheme 1.

Table I. Oxidation of SMX

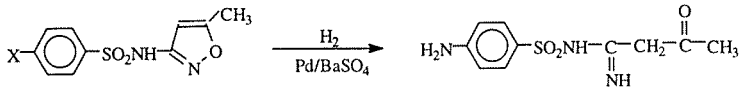
Reaction	SMX/H_2O_2 molar ratio	Solvent	Temperature (°C)	Time (h)	Catalyst	Product
a	1:2	Acetic acid	20	24	–	**1**
b	1:7	Acetic acid	20	46	–	**2**
c	1:7	Acetic acid	100	2	–	**2**
d	1:40	Methanol	40	15	H_2SO_4	**2**
e	1:9	Methanol	20	1.5	MTO	**3**



Scheme 1

Catalytic hydrogenation of SMX was studied as well. Using 5% palladium on $BaSO_4$ as catalyst opening of isoxazole ring occurred and the amidine **4** was obtained in good yield (reaction *f*). The reduction of the nitro compound **2** with the same catalyst led to the product **4** as well (reactions *g* and *h*). The fact that the hydrogenation reaction of the amino compound was performed more slowly than the hydrogenation of the corresponding nitro compound indicated that the ring opening occurred parallel to the reduction of nitro group and intermediate products. The reaction times were prolonged in all hydrogenation reactions in order to remove the traces of the starting compounds. Catalytic hydrogenation of SMX and SMX-NO_2 is shown in Scheme 2.

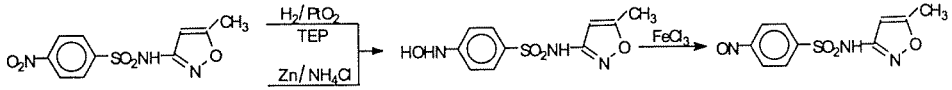
By the use of a poisoned platinum catalyst (PtO_2 plus TEP), compound **2** was reduced to the corresponding hydroxylamine product **5** (SMX-HA) according to the litera-



X = NH_2 or NO_2 4

Scheme 2

ture procedure (4) (reaction *i*). The same product was formed when **2** was treated with zinc dust and aqueous ammonium chloride solution, analogously to the well known reductions of nitro compounds to hydroxylamines (13) (Scheme 3). The catalytic hydrogenation method proved to be more convenient since the product from this reaction was easier to isolate. The oxidation of the SMX-HA to nitroso compound **1** with acidic dichromate solution at 0 °C failed but it was successfully performed by ferric chloride as it was described in the literature (7). The IR and ^{1}H NMR spectra of the as-prepared **1** were identical to the spectra of the compound prepared by SMX selective oxidation (reaction *a*).



2 5 1

Scheme 3

The reaction conditions of catalytic hydrogenation reactions are shown in Table II and hydrogen consumption in Fig. 1.

Table II. Catalytic hydrogenation of SMX or SMX-NO_2 (2)

Reaction	Starting compound	Catalyst	Substrate/catalyst mass ratio	TEP	Solvent	Product
f	1	$Pd/BaSO_4$	1:1	–	Dioxane	4
g	2	$Pd/BaSO_4$	1:1	–	Ethyl acetate	4
h	2	$Pd/BaSO_4$	1:1	–	Dioxane	4
i	2	PtO_2	10:1	+	Ethanol	5

The structures of the compounds were confirmed by IR, NMR and mass spectra. To the best of our knowledge, only the limited spectroscopic data on the synthesized compounds are available in literature. Therefore these data have been added in the Experimental. Products **3** and **4** are new compounds. Azoxy derivative **3** in IR spectrum has two typical absorbtion bands at 3437 (NH) and 1616 (N=N) cm^{-1}. Molecular ion peak in mass spectrum (518) and ^{1}H NMR spectrum cleary indicate the proposed structure. IR spectrum of amidine **4** shows absorption bands at 3377 and 3334 (NH_2), 1711 (CO) and 1637 (C=N). Mass spectrum data ($[M+1]^+$ 256 and $[M-1]^+$ 254), ^{1}H NMR and ^{13}C NMR

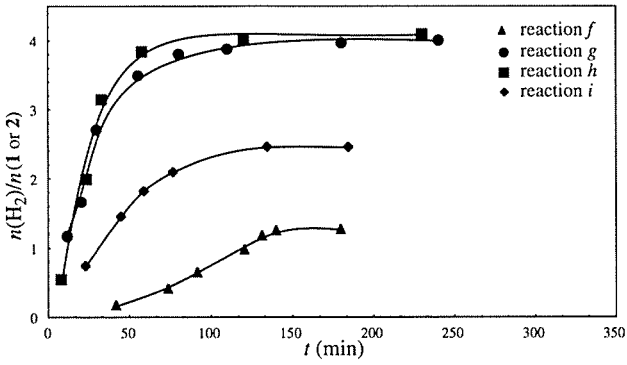


Fig. 1. Consumption of hydrogen in catalytic hydrogenation reactions.

spectra confirm the chemical structure. The structure of **2** was confirmed by comparing the product **2** with the product obtained by direct condensation of 4-NSC and AMI (4).

Abbreviations. – SMX, sulfamethoxazole; SMX-NO, 4-nitroso-*N*-(5-methyl-3-isoxazolyl)benzenesulfonamide; SMX-NO_2, 4-nitro-*N*-(5-methyl-3-isoxazolyl)benzenesulfonamide; SMX-AZ, azoxybenzene-4,4-(*N*-5-methyl-3-isoxazolyl)disulfonamide; SMX-AD, *N*-(4-aminobenzenesulfonyl)-3-oxo--butyramidine; SMX-HA, 4-hydoxylamino-*N*-(5-methyl-3-isoxazolyl)benzenesulfonamide; MTO, merhylrhenium trioxide; 4-NSC, 4-nitrobenzenesulfonyl chloride; AMI, 3-amino-5--methylisoxazole; TEP, triethyl phosphite; DB, *p*-dimethylbenzaldehyde.

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SAŽETAK

Oksidacija i redukcija sulfametoksazola

MONIKA LOVREK, BRANKA ZORC i IVAN BUTULA

Proučavane su reakcije oksidacije i redukcije sulfametoksazola (SMX). Oksidacijom sulfametoksazola pomoću vodikovog peroksida u ledenoj octenoj kiselini dobiven je odgovarajući nitrozo **1** (SMX-NO) ili nitro derivat **2** (SMX-NO_2). Kad je reakcija katalizirana metilrenijevim trioksidom (MTO) kao glavni produkt dobiven je azoksi derivat **3**. Hidrogenacijom SMX uz katalizator Pd/$BaSO_4$ nastao je amidin **4** (SMX-AD). Isti produkt dobiven je katalitičkom hidrogenacijom nitro spoja **2** uz analogne reakcijske uvjete. Hidrogenacija spoja **2** uz katalizator PtO_2 i trietil-fosfit (TEP) dala je hidroksilamin **5** (SMX-HA). Isti produkt dobiven je redukcijom spoja **2** cinkom. Selektivnom oksidacijom spoja **5** priređen je nitrozo spoj **1**.

Ključne riječi: sulfametoksazol, oksidacija, katalitička hidrogenacija

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