Demethylation Reactions of 4-Amino-*N*-(2,6-dimethoxy-4-pyrimidinyl)benzenesulfonamide (Sulfadimethoxine) in Strongly Basic Aqueous Solution

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Hydrolysis of **1** in strongly basic aqueous solution afforded mono- and didemethylated products **2**, **3** and **4**, that are postulated as the metabolites of **1** in some animals. This hydrolytic demethylation was shown to proceed stepwise *via* mono-demethylation to give **2** and **3**, followed by their further demethylation to **4**. The hydrolytic reactivity of **1-3** was rationalized based on MO calculation results and ^{13}C nmr data.

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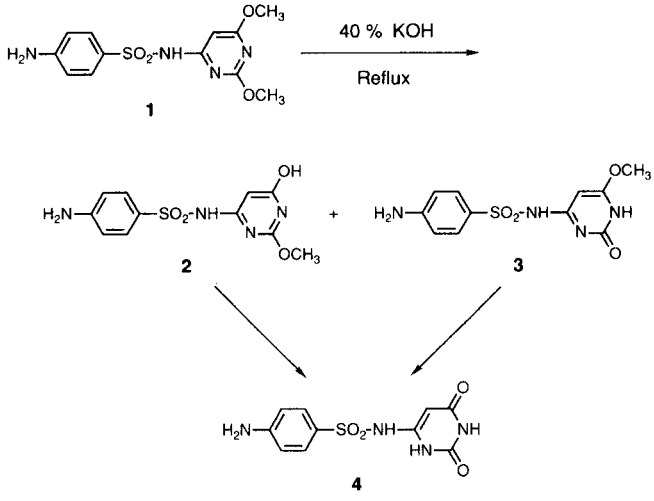
4-Amino-*N*-(2,6-dimethoxy-4-pyrimidinyl)benzenesulfonamide (trivial Sulfadimethoxine **1**) is known as a potent antibiotic agent developed by Hoffmann-La Roche Inc., in 1955 as a lasting sulfonamide for humans as well as animals [1]. The metabolism of such an agent is very important in practical usage. Very recently, mono- and dimethylated compounds of **1**, *i.e.*, compounds **2**, **3** and **4** were postulated as the metabolites of **1** in chickens [2], turtles [3] and snails [4], but their detailed characterization has not been carried out yet to the best of our knowledge.

From above viewpoint as well as reactivity-stability problem of methoxy-substituted pyrimidine derivatives, we studied the demethylation reaction of **1** in strongly basic aqueous solution. The acidic hydrolysis of **1** was known to afford sulfanilamide and barbituric acid [5], while the hydrolysis of **1** under mild alkaline conditions was reported to afford monodemethylated compound **3** accompanied by uncharacterized products [6]. We report herein demethylation of **1** in strongly basic aqueous solution, and the demethylation mechanism on the basis of ^{13}C nmr spectral data and a MO calculation study of **1** and related model compounds.

Heating of **1** under reflux in 40% aqueous potassium hydroxide for 7.5 hours afforded compound **3** in 66% isolated yield, accompanied by minor products **2** and **4**. Compound **4** was also obtained in 32% yield from **3** under similar reaction conditions (Scheme 1). Their structures were confirmed by CHN elemental analysis and spectral data. In the ^1H nmr spectrum, **3** had a characteristic singlet signal at δ 5.82 ppm assignable to C_5-H on the 2-oxopyrimidine nucleus. Previously the 2-oxo structure of **3** was assumed based on 1720 cm^{-1} absorption in ir spectrum [6]. Compound **2** revealed a singlet signal at δ 5.40 ppm due to C_5-H of the 6-hydroxypyrimidine ring. It may be of interest to mention that compound **2** (mp 272-274°) was in fact identical with the metabolic product of **1** in chickens by their ir and nmr spectral data [2], while compound **3** (mp 182-184°) and **4** (mp 248-250°) had the same retention times with the metabolites of **1** in turtles and in snails respectively on hplc analysis [3,4]. Hence, compound **2**, **3** and **4** were concluded to be the metabolites of **1** in these animals.

Scheme 1



The demethylation reaction of **1** was followed by hplc analysis under various conditions as summarized in Table 1: in hot solution of concentrated strong base such as 40% aqueous potassium hydroxide and -sodium hydroxide, the hydrolytic demethylation of **1** proceeded rapidly to afford **2**, **3** and **4** (entry No. 1-2). However, in hot solution of dilut-

Table 1

Demethylation of **1** under Aqueous Basic Conditions

Entry No.	Base concentration	Temperature (°C)	Time (hours)	1 (%)	2 (%)	3 (%)	4 (%)	Unknown [a] (%)
1	40% KOH	120-130	7	0	20.6	59.0	19.3	1.1
2	40% NaOH	120-130	7	0	15.3	58.9	24.1	1.8
3	20% KOH	120-130	7	38.2	13.5	37.2	11.1	0
4	10% KOH	120-130	7	81.3	3.8	11.2	3.7	0
5	5% KOH	120-130	6	98.0	0	2.0	0	0
6	40% KOH	60-70	8	95.1	0	4.9	0	0
7	40% KOH	60-70	72	84.7	2.9	11.3	1.1	0
8	14% $NaOCH_3$-H_2O	110-120	7	93.5	1.0	4.5	1.0	0

[a] Retention time = 2.3 minutes.

Table 2

^{13}C NMR Spectral Data of **1** and Related Compounds

Compounds	R_1	R_2	Positions [a] 2	4	6	Positions [b] 2	4	6
1	OCH_3	OCH_3	171.6	160.1	164.4	174.7	167.5	171.3
	lit [6]		171.5	160.0	164.3			
7	OCH_3	H	157.4	158.7	169.7	160.1	169.5	172.7
5	H	OCH_3	164.5	159.6	158.8	170.1	167.3	158.9
2	OH	OCH_3	164.3	158.3	157.2	168.5	169.1	180.2
8	OH	H	156.8	161.6	150.1	161.0	167.9	178.2
3	OCH_3	=O	148.9	159.9	163.5	172.2	171.7	175.4
	lit [6]		148.9	159.8	163.6			
6	H	=O	149.4	159.7	143.8	172.1	170.5	160.3

[a] Obtained in dimethyl sulfoxide-d_6. [b] Obtained in sodium 5% deuteroxide-deuterium oxide.

ed base, the demethylation became very sluggish (entry No. 3-5). The reaction at 60-70° in strong bases was also very sluggish (entry No. 6-7).

The time-course of the product ratio in 40% aqueous potassium hydroxide at 120-130° is shown graphically in Figure 1: compound **1** was consumed with a life time $\tau_{1/2}$

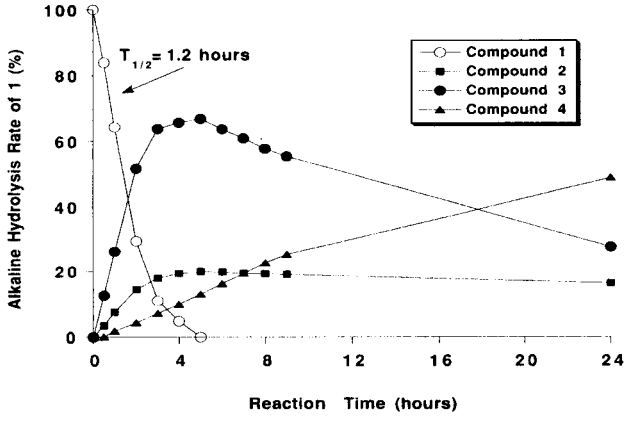


Figure 1. Product ratio in the demethylation of **1** in 40% aqueous potassium hydroxide at 120-130°.

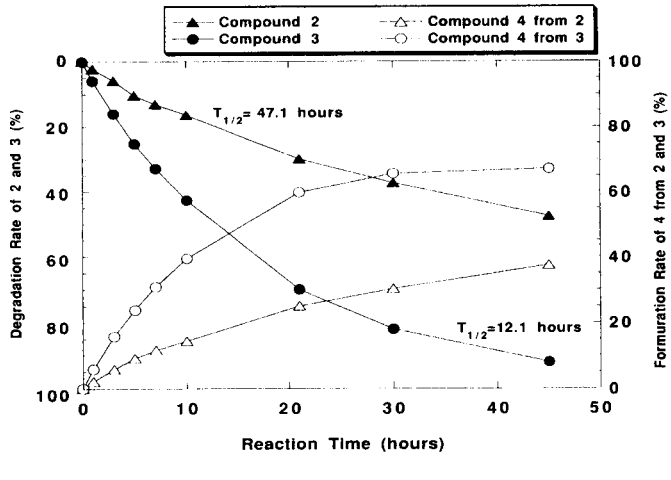


Figure 2. Product ratio of relative degradations of **2** and **3** in 40% aqueous potassium hydroxide at 120-130°.

= 1.2 hours, the yield of major product **3** became the maximum after 5 hours; compounds **2** and **3** decreased gradually and instead, compound **4** was increased gradually; after 18 hours, compound **4** became the major product; the time-course of the independent degradation of **2** and **3** (Figure 2) indicated clearly the reactivity difference between **2** ($\tau_{1/2}$ = 47.1 hours) and **3** ($\tau_{1/2}$ = 12.1 hours); after 45 hours, the conversions of **2** and **3** to **4** were 40 and 70%, respectively. The demethylation of a 1:1 mixture of **2** and **3** under the same conditions gave the same results in indicate no interfering interactions between **2** and **3** in these hydrolytic demethylation reactions.

As above, hydrolytic demethylation of **1** in hot strong base proceeds *via* a concurrent displacement of methoxy groups on the pyrimidine nucleus to afford **2** and **3** in the first step, followed by their further demethylation to **4** with a different reaction rate in the second step.

Semi-empirical MO calculation of 4-aminomethoxypyrimidines A-D as the model compounds of **1-4** was performed to elucidate the observed reactivity as above. The calculated stability energy level and the coefficient LUMO of 2Pz at the C-2 and C-6 positions on the pyrimidine nucleus in suitable conformer are shown below (Figure 3).

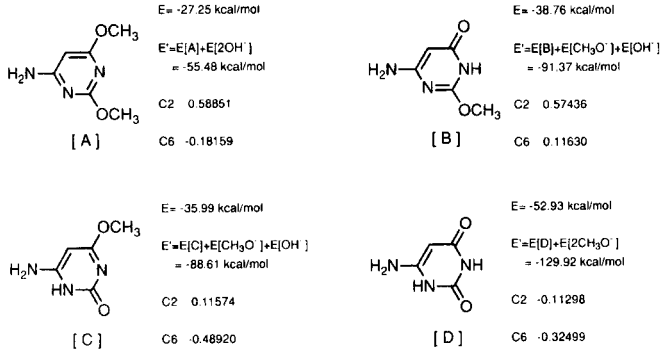
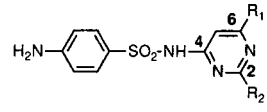


Figure 3. The calculated energy level and the coefficient LUML of A-D.

The calculated stability energy of model compounds is in the order of **D** ≫ **C** > **B** > **A**, and this is in a gross accord with the relative stability of **1-4** in alkaline solution. As for the coefficient LUMO of 2Pz at the C-2 and C-6 positions of **A**, the absolute value of C-2 (0.58851) is larger than that of C-6 (-0.18159), suggesting C-2 position of the pyrimidine nucleus is more reactive than C-6 position for the nucleophilic attack by hydroxyl anion. And hence, the observed rapid hydrolysis of **1** at C-2 (to give **3**) compared with at C-6 (to give **2**) is thus reasonable. But it is not easy to rationalize the reactivity difference between **2** and **3** by the coefficient values of LUMO of 2Pz at C-2 of **B** and C-6 of **C** respectively.

Next we examined ^{13}C nmr data of compounds **1-3**, and related compounds **5-8**. Compound **5** [7] and **6** [8] were synthesized according to literature procedures, and **8** was prepared by the hydrolysis of 4-amino-*N*-(6-methoxy-4-pyrimidinyl)benzenesulfonamide **7** in 10% aqueous sodium hydroxide under reflux. The chemical shift assignments of C-2, C-4 and C-6 of pyrimidine nucleus in these compounds are summarized in Table 2. The assignment of C-2 and C-6 in **2** and **3** was clarified by using COLOC method (see Experimental). The chemical shift of C-2 (174.7 ppm) of **1** appeared at lower field than that of C-6 (171.3 ppm; in sodium deuteroxide-deuterium oxide), indicating the electron density on C-2 position is lower than that of C-6 position [9]. Consequently, C-2 position should be more reactive than C-6 position against the attack by the nucleophilic hydroxyl anion. The C-2 (168.5 ppm) of **2** appeared at higher field than C-6 (175.4 ppm) of **3** in 5% sodium deuteroxide-deuterium oxide, suggesting a higher electron density at C-2 of **2** than C-6 of **3**. This difference may be attributable to the lower reactivity of **2** compared with **3** in the alkaline hydrolysis. Thus, the reactivity difference toward the nucleophilic hydroxyl anion between **2** and **3** can be rationalized on the basis of ^{13}C nmr data.

As above the demethylation of **1-3** in aqueous base proceeds *via* nucleophilic displacement of methoxy group with hydroxyl anion. The reactivity differences are rationalized based on MO calculation results and ^{13}C nmr data. These results clarified the behavior of methoxy-substituted pyrimidine derivatives in strong basic aqueous solution.



EXPERIMENTAL

Melting points were determined on a Yanaco micromelting point apparatus and are uncorrected. The spectra were recorded on the following instruments; ir, Hitachi 270-30 Infrared Spectrophotometer; ^1H and ^{13}C nmr, JEOL JNM-GSX-500 or JNM-EX400 spectrometer; ms, JEOL JMS-HX110 or a JMS-D300 mass spectrometer, using a field-desorption ionization technique. Elemental analysis was performed on a Perkin Elmer 2400 CHN Elemental Analyzer. The hplc analysis was performed on a Shimadzu LC-6AD apparatus.

Isolation of 4-Amino-*N*-(6-hydroxy-2-methoxy-4-pyrimidinyl)benzenesulfonamide (**2**), 4-Amino-*N*-(6-methoxy-2-oxo-4-pyrimidinyl)-benzenesulfonamide (**3**) and 4-Amino-*N*-(2,6-dioxo-4-pyrimidinyl)-benzenesulfonamide (**4**).

A mixture of **1** (20 g, 64 mmoles) in 40% aqueous potassium hydroxide (100 ml) was refluxed for 7.5 hours and the ice-cooled reaction mixture was neutralized with concentrated hydrochloric acid (30 ml) and acetic acid (20 ml) to *p*H 6-7. The resulting precipitate was filtered, washed with water (10 ml), dried and extracted with hot ethanol, repeatedly (600 ml x 4). The combined

ethanol extracts were concentrated to about one fourth *in vacuo*, and the resulting precipitate was filtered and washed with water. Recrystallization from 80% aqueous ethanol (1 *l*) gave **2** as colorless crystal, 2.0 g (11%), mp 272-274°; ir (potassium bromide): 3512, 3408 (NH) cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 11.99 (s, 1H, SO_2NH), 10.78 (br s, 1H, OH), 7.54 (d, J = 9 Hz, 2H, phenyl), 6.61 (d, J = 9 Hz, 2H, phenyl), 6.11 (br s, 2H, NH_2), 5.40 (s, 1H, pyrimidine 5-H), 3.79 (s, 3H, 2-OCH_3); ms: m/z 297 ($M^+ + 1$).

Anal. Calcd. for $C_{11}H_{12}N_4O_4S$: C, 44.59; H, 4.08; N, 18.91; S, 10.82. Found: C, 44.57; H, 4.04; N, 18.93; S, 10.72.

The extraction residue was extracted by 50% aqueous ethanol (600 ml x 4) again, and concentrated to about one fourth *in vacuo*, and the resulting precipitate was filtered and stirred in water (120 ml) for 30 minutes and filtered. The filtrate was dried up *in vacuo* to give crude potassium salt of **4** (about 4 g), and this salt was dissolved into water (90 ml) and acidified to *p*H 3-4 with 1*N* hydrochloric acid. The precipitate was filtered, washed with water, and crystallized from 50% ethanol to yield 0.75 g of **4** as colorless crystals, mp 248-250°; ir (potassium bromide): 3384 (NH), 1714 (C=O), 1634 (NH), 1192 (SO_2) cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 10.87 (s, 1H, SO_2NH), 7.51 (d, J = 9 Hz, 2H, phenyl), 6.64 (d, J = 9 Hz, 2H, phenyl), 4.98 (s, 1H, pyrimidine 5-H); ms: m/z 282 (M^+).

Anal. Calcd. for $C_{10}H_{10}N_4O_4S$: C, 42.55; H, 3.57; N, 19.85; S, 11.36. Found: C, 42.86; H, 3.63; N, 19.78; S, 11.09.

The mother liquor of the 50% aqueous ethanol extraction was dried up *in vacuo* to give the potassium salt of **3** (3.7 g). Also, the residue of the water extraction was washed with 50% aqueous ethanol to yield the potassium salt of **3** (6.9 g, total 10.6 g, mp >300°). This salt (1.2 g) was dissolved in 1*N* hydrochloric acid (5.4 ml) and 20% aqueous ethanol (64 ml) stirred at 80-90° for 30 minutes, and the solution was cooled to 10-20°. The precipitate was collected and recrystallized from 20% aqueous ethanol (50 ml) to afford 0.70 g (66%) of **3** as colorless crystals, mp 182-184° (lit [6] 179-180°); ir (potassium bromide): 3476, 3388 (NH), 1720 (C=O), 1634, 1186, 1088 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 11.42 (s, 1H, SO_2NH), 7.48 (d, J = 9 Hz, 2H, phenyl), 6.60 (d, J = 9 Hz, 2H, phenyl), 5.82 (s, 1H, pyrimidine 5-H), 3.85 (s, 3H, 6-OCH_3); ms: m/z 296 (M^+).

Anal. Calcd. for $C_{11}H_{12}N_4O_4S$: C, 44.59; H, 4.08; N, 18.91; S, 10.82. Found: C, 44.24; H, 4.10; N, 18.73; S, 10.59.

On the other hand, refluxing of **3** (1.0 g) in 40% aqueous potassium hydroxide (5 ml) for 13 hours also gave **4** of 0.3 g (32%) after the similar work up.

4-Amino-*N*-(2-methoxy-4-pyrimidinyl)benzenesulfonamide (**5**).

A mixture of 4-acetylamino-*N*-(2-methoxy-4-pyrimidinyl)benzenesulfonamide [7] (5.0 g, 18 mmoles) in 1*N* sodium hydroxide (60 ml) was stirred for 3 hours at 80-90°. After cooling, the reaction solution was made slightly acidic by acetic acid. The precipitate was collected and crystallized from 50% aqueous ethanol to yield 2.1 g (48%) of **5** as colorless crystal, mp 187-189° (lit [7] 187-188°); ir (potassium bromide): 3476, 3380 (NH_2, NH), 1368 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 11.32 (br s, 1H, NH), 8.22 (d, J = 6 Hz, 1H, pyrimidine 6-H), 7.58 (d, J = 9 Hz, 2H, phenyl), 6.59 (d, J = 9 Hz, 2H, phenyl), 6.59 (d, J = 6 Hz, 1H, pyrimidine 5-H), 6.10 (s, 2H, NH_2), 3.79 (s, 3H, OCH_3); ms: m/z 281 ($M^+ + 1$), 216 ($M^+ - SO_2$).

Anal. Calcd. for $C_{11}H_{12}N_4O_3S$: C, 47.13; H, 4.32; N, 19.99. Found: C, 47.00; H, 4.32; N, 19.96.

4-Amino-*N*-(2-oxo-4-pyrimidinyl)benzenesulfonamide (**6**).

This compound was prepared from demethylation of **5** in acidic conditions [8].

A mixture of **5** (420 mg, 1.5 mmoles) in 10% aqueous hydrogen iodide (3.0 ml) was stirred for 1 hour at 80-85°. After cooling, the solution was neutralized by 10% aqueous sodium hydroxide, and the precipitate was filtered and washed with water. The solid was dissolved in 1% aqueous sodium hydroxide (2 ml), and then acidified (*p*H 5.0) with acetic acid at room temperature. The precipitate was collected and recrystallized from 50% aqueous methanol to afford **6** as pale yellow crystals (290 mg, 73%), dec 272-282° (lit [8] dec 270°); ir (potassium bromide): 3480, 3376 (NH_2, NH), 1634 (C=O), 1392 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 11.61 (br s, 2H, NH, CONH), 7.55 (d, J = 8 Hz, 1H, pyrimidine 6-H), 7.43 (d, J = 9 Hz, 2H, phenyl), 6.58 (d, J = 9 Hz, 2H, phenyl), 6.37 (d, J = 8 Hz, 1H, pyrimidine 5-H), 5.88 (s, 2H, NH_2); ms: m/z 267 ($M^+ + 1$).

Anal. Calcd. for $C_{10}H_{10}N_4O_3S$: C, 45.11; H, 3.79; N, 21.04. Found: C, 45.28; H, 3.75; N, 20.91.

Also, **6** was obtained in 24% yield from alkaline hydrolysis of **5** in 10% aqueous sodium hydroxide under refluxing for 7.5 hours.

4-Amino-*N*-(6-hyroxy-4-pyrimidinyl)benzenesulfonamide (**8**).

A mixture of 4-amino-*N*-(6-methoxy-4-pyrimidinyl)benzenesulfonamide **7** ([1] 5.0 g, 18 mmoles) in 10% aqueous sodium hydroxide (100 ml) was heated at reflux with stirring for 14 hours. After cooling, the reaction mixture was poured into water (50 ml) and acidified with acetic acid (25 ml). The precipitate was filtered, washed with water, and dried. The solid was refluxed in acetone (120 ml) for 30 minutes to remove raw material, and filtered. The residue (4.48 g) was refluxed in 50% aqueous ethanol (800 ml) for 1 hour, filtered, and the filtrate was concentrated *in vacuo* to about one third volume. After standing for one day, the precipitate was filtered and crystallized from 50% aqueous ethanol to yield 0.75 g (16%) of **8** as a pale yellow powder, mp >300°; ir (potassium bromide): 3492, 3368 (NH_2, NH), 1658, 1622 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 11-12 (br s, 1H, NH), 7.98 (d, J = 6 Hz, 1H, pyrimidine 2-H), 7.52 (d, J = 9 Hz, 2H, phenyl), 6.61 (d, J = 9 Hz, 2H, phenyl), 5.72 (s, 1H, pyrimidine 5-H), 6.13 (br s, 2H, NH_2); ms: m/z 267 ($M^+ + 1$), 202 ($M^+ - SO_2$).

Anal. Calcd. for $C_{10}H_{10}N_4O_3S$: C, 45.11; H, 3.79; N, 21.04. Found: C, 45.09; H, 3.93; N, 20.82.

HPLC Analysis of Alkaline Hydrolysis Products.

The change of alkaline hydrolysis products was followed by the hplc method. The sample for hplc injection was prepared by the following procedure: after alkaline hydrolysis, 0.5 ml of the reaction mixture was diluted with water to 100 ml, and 0.5 ml of this solution was diluted to 100 ml again, and injected (5μl); column: reversed phase column (ODS type, ϕ 4.6 x 150 mm), mobile phase 1/200 *M* ammonium acetate, acetic acid buffer: acetonitrile = 7:3 (v/v), 1.0 ml/minute, detection uv 274 nm. The retention times of **1**, **2**, **3** and **4** were 8.0, 2.5, 2.1 and 1.7 minutes, respectively.

Measurement of ^1H-^{13}C COLOC Spectra.

The ^1H-^{13}C COLOC (Correlation Spectroscopy *via* Long-Range Couplings) spectra were measured (observation: 5 Hz, 10 Hz) in 5 wt% sodium deuteroxide-deuterium oxide on a JEOL JNM-GSX-500 (500 MHz) spectrometer.

MO Calculations of Model Compounds.

The MO calculations were carried out on a FACOM M760/10 computer using the MOPAC program (version 5.0 [10]). The

geometries of the 4-amino-2,6-dimethoxypyrimidines for the semi-empirical MO calculations (AM1 method) were estimated from values based on the standard bond lengths and angles [11].

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