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Kinetics and Mechanism of the Acid–Base Equilibrium of Mexazolam and Comparison with Those of Other Commercial Benzodiazepinooxazole Drugs[1)]

YUKIHISA KURONO,*,[a] KINYA KAMIYA,[a] TOMONARI KUWAYAMA,[b] YASUHIRO JINNO,[a] TAMOTSU YASHIRO,[a] and KEN IKEDA[a]

Faculty of Pharmaceutical Sciences, Nagoya City University,[a] 3–1 Tanabe-dori, Mizuho-ku, Nagoya 467, Japan and Pharmacy, Tokai Teishin Hospital,[b] 2–17–5 Matsubara, Naka-ku, Nagoya 460, Japan

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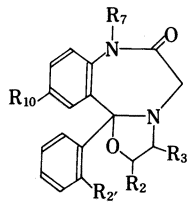
The oxazolidine ring-opening and ring-closing reactions of mexazolam, cloxazolam, haloxazolam, and flutazolam were investigated by a pH-jump method, similarly to the case of oxazolam reported previously [Kurono *et al.*, *Chem. Pharm. Bull.*, **33**, 1633 (1985)]. Mexazolam exists essentially as a single isomer, either *cis* or *trans* (referring to the 3-methyl group and 11b-(2′-chlorophenyl) group), differently from the case of oxazolam (*cis* isomer/*trans* isomer ratio for oxazolam ≒ 1 : 1). Over the pH range of 1—13, the pH–rate profiles show two step reactions. For interpretation of these profiles, we propose a reaction mechanism including an intermediate, detected by the kinetic method, between the iminium structure (oxazolidine ring-opened form) and the ring-closed form. These kinetic properties of mexazolam differ from those of other benzodiazepinooxazoles, and the difference is caused by the presence of the 3-methyl group rather than the 2′-chlorine atom. The intrinsic rate constants of mexazolam, its 2′-chlorine deficient analog (3-methyl compound), cloxazolam, haloxazolam, and flutazolam were determined according to the appropriate reaction schemes.

Keywords——mexazolam; cloxazolam; haloxazolam; flutazolam; benzodiazepinooxazole; oxazolidine ring-opening, -closing; kinetics; *cis–trans* isomer; acid–base equilibrium; pH–rate profile

In the previous paper[2)] we reported the kinetics and mechanism of the oxazolidine ring-opening and ring-closing reactions (acid–base equilibrium) of oxazolam. Rate measurements using a stopped-flow spectrophotometer allowed us to distinguish between the *cis* and *trans* isomers (referring to the 2-methyl group and 11b-phenyl group). Mexazolam is also considered to possess *cis* and *trans* isomers (referring to the 3-methyl group and 11b-(2′-chlorophenyl) group). However, when the rates of the ring-opening and -closing reactions of mexazolam were examined, the kinetic behavior of mexazolam was found to be very different from that of oxazolam and other benzodiazepinooxazoles.

In this paper we describe at first the kinetic results for cloxazolam, haloxazolam, and flutazolam (commercial benzodiazepinooxazole drugs) for easy understanding of the difference in the reaction mechanism between mexazolam and other benzodiazepinooxazoles. Then we propose a reaction mechanism for the rate process of the acid–base equilibrium of mexazolam. The results for the 2′-chlorine deficient analog of mexazolam (3-methyl compound) synthesized are also presented. The chemical structures of the drugs used are shown in Chart 1.



	R_2	$R_{2'}$	R_3	R_7	R_{10}
cloxazolam	H	Cl	H	H	Cl
haloxazolam	H	F	H	H	Br
flutazolam	H	F	H	C_2H_4OH	Cl
mexazolam	H	Cl	CH_3	H	Cl
3-methyl compound	H	H	CH_3	H	Cl
oxazolam	CH_3	H	H	H	Cl

Chart 1

Experimental

Materials and Instruments——Mexazolam (lot No. 2), cloxazolam (lot No. 2), and haloxazolam (lot No. 6) were supplied by Sankyo Co., Ltd. Flutazolam (lot No. A 424490) was a gift from Mitsui Pharmaceutical Co., Ltd. All the drugs were used without further purification. The 3-methyl compound was synthesized by a procedure similar to those reported by Deriege *et al.*,[3] Miyadera *et al.*,[4] and Lemke and Hanze.[5] All other chemicals were purchased commercially and were of reagent grade.

Ultraviolet (UV) absorption spectroscopy was carried out with a Hitachi UV-124 spectrophotometer and a Shimadzu UV-260 spectrophotometer. The reaction rates were measured with a Hitachi UV-124 spectrophotometer. ^{1}H- and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra were obtained with a JEOL JNM-FX 100 spectrometer at 100 and 25 MHz, respectively, using tetramethylsilane as an internal standard. A Hitachi-Horiba F-7_{LC} pH meter was used for pH measurement. An NEC microcomputer (PC-9801E) was employed for the calculation of rate constants and equilibrium constants and for the simulation of the pH–rate profiles.

Determination of Equilibrium Constant——The apparent equilibrium constants and dissociation constants of benzodiazepinooxazoles were determined by methods similar to those reported previously.[2,6]

Kinetic Procedures——The buffer systems were the same as those used in the previous studies.[2] The UV spectra of mexazolam at pH 3—5 were measured in 0.033 M glycine–HCl buffer and 0.017 M acetate buffer (one third or one sixth of the ordinary buffer concentrations) because 0.1 M glycine and 0.1 M acetate buffers show significant absorbances below 230 nm and disturb the spectra of mexazolam below 230 nm.

Rate measurements of the oxazolidine ring-opening and ring-closing were carried out by the pH-jump method as reported previously.[2] In addition to the stopped-flow method, the conventional UV method was also applied for the slow rate measurements of mexazolam and the 3-methyl compound. All the experiments except for the ^{1}H-NMR and ^{13}C-NMR measurements of mexazolam and the 3-methyl compound were carried out at 25 °C in aqueous buffer containing 4% (v/v) ethanol with μ=0.1 M (NaCl).

Results and Discussion

pH–Rate Profile for Acid–Base Equilibrium of Benzodiazepinooxazoles

Figure 1 illustrates the UV spectra of cloxazolam in various pH buffer solutions. These spectra are attributable to equilibrium mixtures of the ring-opened iminium form (AF) in acid solution and the ring-closed form (BF) in weakly alkaline solution (see Chart 2 for structures).[2-4] From the spectral data, similarly to the case of oxazolam studied previously,[2] the apparent pK_{eq} value ($-\log([BF][H^+]/[AF])$) and the $pK_{a,2}$ value ($-\log([BA][H^+]/[BF])$) were estimated as 6.90 and 10.8, respectively. These values agree fairly well with those in the literature.[7-9]

Figure 2 shows the pH–rate profiles for the acid–base equilibria of cloxazolam and flutazolam. The shape of the profile for cloxazolam is similar to that for oxazolam reported previously,[2] suggesting that the reaction scheme shown in Chart 2 is applicable. In Chart 2 $K'_{a,2}$ and $K_{a,2}$ are the dissociation constants of AF and BF, respectively, these processes being much faster than the processes of ring-opening and -closing.[2] The presence of the processes corresponding to $K'_{a,2}$ and $K_{a,2}$ is supported by the absence of the inflection at the weakly alkaline region in the pH–rate profile of flutazolam and by the absence of the spectral change with the strongly alkaline flutazolam solutions, respectively. Flutazolam carries a 2-

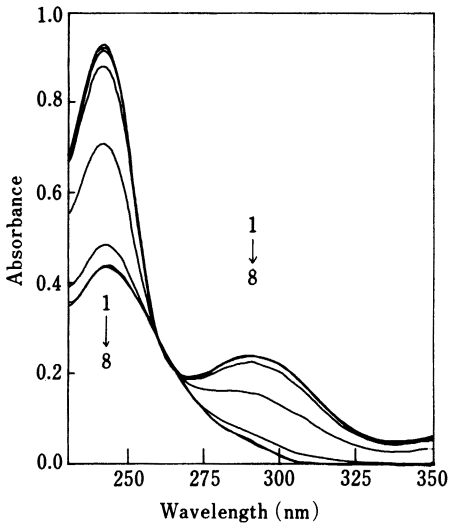


Fig. 1. UV Absorption Spectra of Cloxazolam at Various pH Values

Concentration of cloxazolam, 3.00×10^{-5} M.
1, pH 3.03; 2, 3.94; 3, 5.01; 4, 6.00; 5, 7.00; 6, 8.02; 7, 9.00; 8, 9.99.

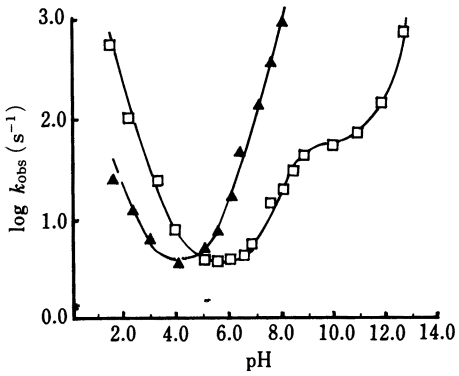


Fig. 2. The pH–Rate Profiles for Oxazolidine Ring-Opening and Ring-Closing Reactions of Cloxazolam and Flutazolam at 25 °C

□, cloxazolam; ▲, flutazolam.

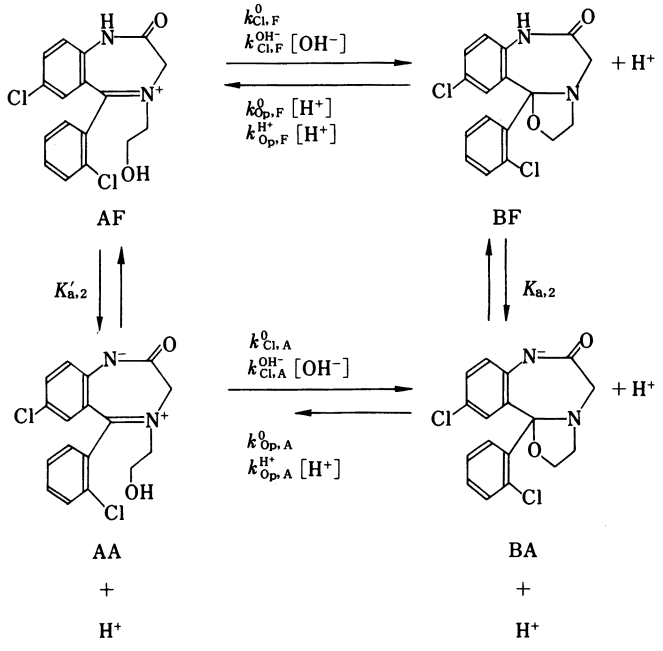


Chart 2

hydroxyethyl group instead of the dissociative hydrogen atom at position 7. In Chart 2 the rate constants represent the following reactions: $k^0_{Cl,F}$, water-catalyzed ring-closing from the free form (AF) of nitrogen at position 7; $k^{OH^-}_{Cl,F}$, hydroxide ion-catalyzed ring-closing; $k^{H^+}_{Op,F}$, hydrogen ion-catalyzed ring-opening from the free form (BF) of the nitrogen; $k^0_{Op,F}$, water-

TABLE I. Estimated Rate Constants and Equilibrium and Dissociation Constants for Cloxazolam, Haloxazolam, and Flutazolam[a)]

	$k^{H^+}_{Op,F}$ $s^{-1}M^{-1}$	$k^0_{Op,F}+k^0_{Cl,F}$ s^{-1}	$k^{OH^-}_{Cl,F}$ $s^{-1}M^{-1}$	$k^0_{Cl,A}$ s^{-1}	$k^{OH^-}_{Cl,A}$ $s^{-1}M^{-1}$	$K'_{a,2}$ M^{-1} ($pK'_{a,2}$)	$K_{a,2}$ M^{-1} ($pK_{a,2}$)	K_{eq} M^{-1} (pK_{eq})
Cloxazolam	2.72×10^4	3.97	2.27×10^7	1.05×10^{-1}	1.62×10^4	3.61×10^{-9} (8.44)	1.58×10^{-11} (10.8)	1.26×10^{-7} (6.90)
Haloxazolam	2.55×10^4	2.06	1.55×10^8	8.25×10^{-1}	7.07×10^4	4.02×10^{-9} (8.94)	2.00×10^{-12} (11.7)	7.94×10^{-7} (6.10)
Flutazolam	2.03×10^3	3.92	1.21×10^9	—	—	—	—	2.51×10^{-6} (5.60)

a) 25 °C; containing 4% (v/v) ethanol.

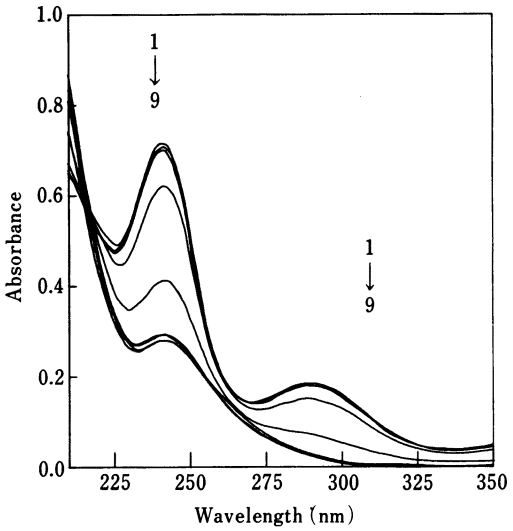


Fig. 3. UV Absorption Spectra of Mexazolam at Various pH Values

Concentration of mexazolam, 3.00×10^{-5} M. 1, pH 3.17[a)]; 2, 4.04[a)]; 3, 5.06[a)]; 4, 6.00; 5, 7.00; 6, 8.02; 7, 9.00; 8, 9.99; 9, 10.99.
a) The concentrations of the buffer constituents were one third or one sixth of the ordinary buffer concentrations used for the kinetic experiments.

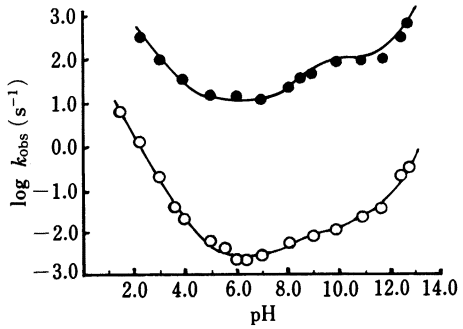


Fig. 4. The pH–Rate Profiles for Oxazolidine Ring-Opening and Ring-Closing Reactions of Mexazolam at 25 °C

●, fast step reaction; ○, slow step reaction.

catalyzed ring-opening; $k^0_{Cl,A}$, water-catalyzed ring-closing from the anionic form (AA) of the nitrogen; $k^{OH^-}_{Cl,A}$, hydroxide ion-catalyzed ring-closing; $k^{H^+}_{Op,A}$, hydrogen ion-catalyzed ring-opening from the anionic form (BA) of the nitrogen; $k^0_{Op,A}$, water-catalyzed ring-opening.

The rate constants and dissociation constants in Chart 2 were determined by methods similar to those used in the case of oxazolam reported previously.[2)] The obtained values, together with the pK_{eq} value, are summarized in Table I. The solid curves in Fig. 2 were calculated by applying the values in Table I to equations which were derived in the way similar to that used for oxazolam.[2)] The differences in the hydroxide ion-catalyzed ring-closing rate constants ($k^{OH^-}_{Cl,F}$ and $k^{OH^-}_{Cl,A}$) between cloxazolam and haloxazolam may reflect the difference in the steric effect, rather than in the electronegativity, between the chlorine atom and fluorine atom at position 2'.[10)]

Kinetics and Mechanism of Acid–Base Equilibrium of Mexazolam

The UV spectral changes with pH for mexazolam are shown in Fig. 3. The analysis of the spectra, done in the same way as for other benzodiazepinooxazoles, gave the pK_{eq} value of 6.90, in fair agreement with the literature value of 6.60.[7]

Figure 4 shows the pH–rate profile for the acid–base equilibrium of mexazolam. Unlike other benzodiazepinooxazoles, two step reactions (fast step ($k_{obs,F}$, ●) and slow step ($k_{obs,S}$, ○)) are found over the whole pH range. The ratio of $k_{obs,F}$ to $k_{obs,S}$ ranges from 10^3 to 10^4. For the ring-opening reaction (initiated by pH-jump, for example, from pH 9.0 to 4.0), the ratio of the absorbance increment at 240 nm due to the fast step ($k_{obs,F}$) to that due to the slow step ($k_{obs,S}$) was about 1 : 6. For the ring-closing reaction (initiated by pH-jump, for example, from pH 3.0 to 9.0), on the other hand, the ratio of the absorbance decrement based on $k_{obs,F}$ to that based on $k_{obs,S}$ was about 4 : 1. From these results of the rate ratio and the absorbance ratio, we propose the reaction mechanism shown in Chart 3 in order to explain the pH–rate profiles for mexazolam. The reaction scheme involves an intermediate (IF) detected by the kinetic method. The absorbance changes due to the reaction process of AF⇄IF are larger than those due to IF⇄BF. The interrelation of the rates, *i.e.*, fast step or slow step, is denoted in Chart 3.

As has been reported previously,[2] oxazolam showed two pH–rate profiles in the acid region due to the *cis* and *trans* isomers (BF_{cis} and BF_{trans}, referring to the 2-methyl group and 11b-phenyl group). The profiles were analyzed according to the reaction scheme shown in Chart 4.[2] For the following reasons, we chose the reaction scheme shown in Chart 3 rather than that in Chart 4 for mexazolam: (1) the ^{1}H-NMR spectrum of mexazolam in methanol-d_4 indicates the existence of essentially only one species, and the ^{13}C-NMR spectrum in chloroform-*d* shows a single signal at δ 97.5 assigned to the carbon atom at position 11b[11]; (2) by using the reaction scheme shown in Chart 4 the ratio of $k_{obs,F}$ to $k_{obs,S}$ can not be related reasonably with the ratio of the absorbance change from the fast step to that from the slow step; (3) two step reactions are observed over the whole pH range for the acid–base equilibrium of mexazolam.

In order to determine whether the 2′-chlorine atom or the 3-methyl group of mexazolam causes the difference in the reaction mechanism between mexazolam (Chart 3) and oxazolam (Chart 4), the 3-methyl compound (see Chart 1 for the chemical structure) was synthesized

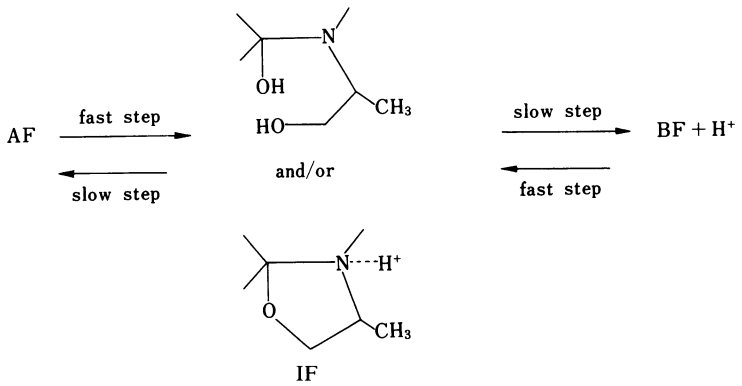


Chart 3

$$H^+ + BF_{cis} \rightleftharpoons AF \rightleftharpoons BF_{trans} + H^+$$

Chart 4

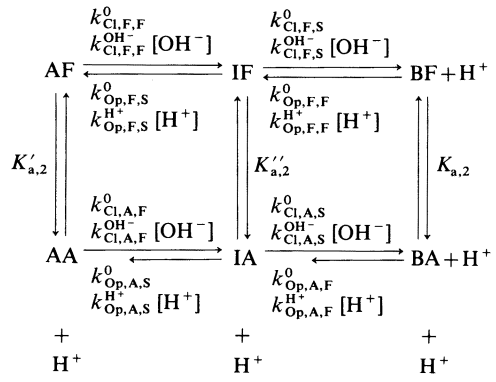


Chart 5

TABLE II. Estimated Rate Constants and Dissociation Constants for Mexazolam and the 3-Methyl Compounds[a]

	$k^{H^+}_{Op,F,F}$ $s^{-1}M^{-1}$	$k^{0}_{Op,F,F}+k^{0}_{Cl,F,F}$ s^{-1}	$k^{OH^-}_{Cl,F,F}$ $s^{-1}M^{-1}$	$k^{0}_{Cl,A,F}$ s^{-1}	$k^{OH^-}_{Cl,A,F}$ $s^{-1}M^{-1}$	$K'_{a,2}$ M^{-1} ($pK'_{a,2}$)	$K_{a,2}$ M^{-1} ($pK_{a,2}$)	K_{eq} M^{-1} (pK_{eq})
Mexazolam	5.36×10^{5}	1.15×10	1.20×10^{7}	1.69	1.43×10^{4}	8.55×10^{-10} (9.07)	6.31×10^{-13} (12.2)	1.26×10^{-7} (6.9)
3-Methyl compound	8.12×10^{4}	2.35	1.30×10^{8}	Negligible	4.40×10^{3}	1.10×10^{-8} (7.96)	1.00×10^{-12} (12.0)	6.31×10^{-7} (6.2)

	$k^{H^+}_{Op,F,S}$ $s^{-1}M^{-1}$	$k^{0}_{Op,F,S}+k^{0}_{Cl,F,S}$ s^{-1}	$k^{OH^-}_{Cl,F,S}$ $s^{-1}M^{-1}$	$k^{0}_{Cl,A,S}$ s^{-1}	$k^{OH^-}_{Cl,A,S}$ $s^{-1}M^{-1}$	$K''_{a,2}$ M^{-1} ($pK''_{a,2}$)
Mexazolam	1.93×10^{2}	2.87×10^{-3}	2.97×10^{3}	1.98×10^{-3}	7.43	1.94×10^{-9} (8.72)
3-Methyl compound	1.82×10^{2}	1.76×10^{-3}	5.09×10^{4}	Negligible	1.00×10	5.03×10^{-8} (7.30)

a) 25 °C; containing 4% (v/v) ethanol.

and kinetic studies were carried out. The pH–rate profiles for the 3-methyl compound were very similar to those for mexazolam, indicating the cause of the difference to be the 3-methyl group rather than the 2′-chlorine atom. The pH–rate profile for cloxazolam, which contains a 2′-chlorine atom and lacks the 3-methyl group (Fig. 2) (one step reaction), also supports the hypothesis that the 3-methyl group of mexazolam is the cause of the difference in the mechanism.

The reaction scheme shown in Chart 5 can account for the pH–rate profiles of mexazolam illustrated in Fig. 4. The superscripts and subscripts of the rate constants in Chart 5 have the following meanings. The superscripts 0, H^+, and OH^- represent the water-catalyzed, hydrogen ion-catalyzed, and hydroxide ion-catalyzed reactions, respectively. The first subscript indicates whether ring-opening (Op) or ring-closing (Cl) occurs, the second (intermediate) one indicates the free form (F) at the 7 nitrogen atom of mexazolam or the anionic form (A), and the last one indicates the fast reaction (F) or the slow reaction (S). $K''_{a,2}$ is the dissociation constant of IF.

According to Chart 5, the apparent first order rate constants for the fast reaction $k_{obs,F}$ and for the slow reaction $k_{obs,S}$ are given by Eqs. 1 and 2, respectively.

$$k_{obs,F}=k^{H^+}_{Op,F,F}[H^+]+k^0_{Op,F,F}+\{1/([H^+]+K'_{a,2})\}\times\{k^0_{Cl,F,F}[H^+]+k^{OH^-}_{Cl,F,F}K_W+k^0_{Cl,A,F}K'_{a,2}+k^{OH^-}_{Cl,A,F}(K_W/[H^+])K'_{a,2}\} \quad (1)$$

$$k_{obs,S}=k^{H^+}_{Op,F,S}[H^+]+k^0_{Op,F,S}+\{1/([H^+]+K''_{a,2})\}\times\{k^0_{Cl,F,S}[H^+]+k^{OH^-}_{Cl,F,S}K_W+k^0_{Cl,A,S}K''_{a,2}+k^{OH^-}_{Cl,A,S}(K_W/[H^+])K''_{a,2}\} \quad (2)$$

By procedures similar to those employed in the case of oxazolam, each parameter was estimated and is listed in Table II. The solid curves in Fig. 4 were simulated by using these parameters. Comparing the results for mexazolam and the 3-methyl compound, it is clear that the 2′-chlorine atom greatly affects the hydroxide ion-catalyzed ring-closing reactions ($k^{OH^-}_{Cl,F,F}$ and $k^{OH^-}_{Cl,F,S}$).

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