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Kinetics of reversible reactions of ampicillin with various aldehydes and ketones with formation of 4-imidazolidinones

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Summary

The reaction of ampicillin with various aldehydes and ketones has been studied in aqueous solution at 37°C. A reversible 4-imidazolidinone formation occurred through condensation between the carbonyl compounds and the α-aminoamide side-chain function of ampicillin. Equilibrium constants and rate constants for the formation and hydrolysis of the imidazolidinones were obtained at pH 7.45. The rate of imidazolidinone formation was found to be depressed by increasing steric effects within the carbonyl compounds whereas the rate of hydrolysis of the imidazolidinones showed only a small dependence on the carbonyl component except for the compound derived from formaldehyde. This derivative showed a half-life of hydrolysis of 29 h at pH 7.45 whereas the imidazolidinones derived from other aldehydes and ketones had half-lives of 4–31 min. The results obtained are discussed in relation to the possible in vivo formation of imidazolidinones by reaction of ampicillin with biogenic aldehydes or ketones and in relation to the possible utility of 4-imidazolidinones as prodrug forms for peptides containing an α-aminoamide moiety.

Introduction

Compounds containing an α-aminoamide moiety are known to condense with aldehydes and ketones giving 4-imidazolidinones (Scheme 1). Davis and Levy (1951) described the condensation of acetone with α-phenylglycineamide to yield 2,2-di-

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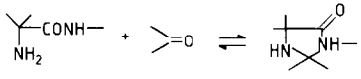
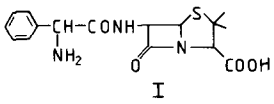


Scheme 1

methyl-5-phenyl-4-imidazolidinone and later, the synthesis of a number of 4-imidazolidinones derived from various dipeptides and ketones or aldehydes was reported (Zehavi and Ben-Ishai, 1961; Yamashiro and du Vigneaud, 1968; Hruby et al., 1968; Panetta and Pesh-Imam, 1972; Ariyoshi and Sato, 1972; Hardy and Samworth, 1977). 4-Imidazolidinones were also reported to be formed by condensation of acetaldehyde with various oligopeptides (Cardinaux and Brenner, 1973), by reaction of acetone with the tripeptide L-propyl-L-leucyl-glycineamide (Hruby et al., 1968) and oxytocin (Yamashiro et al., 1965; Hruby et al., 1968) and by reaction of acetaldehyde with various enkephalins and β-endorphin (Summers et al., 1980; Summers and Lightman, 1981). Furthermore, 4-imidazolidinones derived from carbonyl compounds and α-aminoanilides have been reported (Katsura, 1971; Katsura and Sugiyama, 1971; Katsura et al., 1971; Nelson et al., 1973). Interestingly, the study by Nelson et al. (1973) showed the in vivo formation of a 4-imidazolidinone derivative from reaction of the key metabolite of lidocaine, N-deethyllidocaine, with acetaldehyde formed by ingestion of alcohol. Finally, the α-aminoamide side-chain in ampicillin (I) is known to react easily with acetone yielding hetacillin (II) (Hardcastle et al., 1966) or with other ketones or aldehydes to give the corresponding 4-imidazolidinones (Johnson and Panetta, 1965). Hetacillin is clinically used as an ampicillin prodrug as it is readily hydrolyzed to ampicillin in aqueous solution, the half-life being 15–20 min at pH 4–8 and 35°C (Tsuji and Yamana, 1974) and about 11 min in vivo as determined after intravenous administration in man (Jusko and Lewis, 1973; Jusko et al., 1973). An advantage of hetacillin is its higher stability in concentrated aqueous solutions (Schwartz and Hayton, 1972; Tsuji and Yamana, 1974) compared with ampicillin sodium which undergoes a facile intermolecular aminolysis by attack of the side-chain amino group in one molecule on the β-lactam moiety of a second molecule (Bundgaard, 1976).

In a previous paper (Klixbüll and Bundgaard, 1984) the hydrolysis kinetics of 4-imidazolidinones derived from acetone and various dipeptides were studied and it was suggested that 4-imidazolidinones may be potentially useful prodrug forms for peptides containing an α-aminoamide function with the aim of solving delivery problems for peptide drugs. For the further evaluation of 4-imidazolidinones as a prodrug type it is, however, of importance to examine the effect of the carbonyl component on the reactivity of the derivatives. To this end, we have chosen ampicillin as a model of a peptide containing an α-aminoamide moiety and studied the kinetics of its reversible reaction with various aldehydes and ketones in aqueous solution. Besides by being of interest for the prodrug studies the present work may be pertinent to the possible in vivo condensation of ampicillin with biogenic carbonyl compounds such as formaldehyde, acetaldehyde and pyruvic acid.



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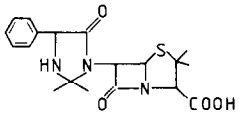


II

Materials and Methods

Chemicals

Ampicillin sodium, amoxycillin trihydrate and cephalexin monohydrate, all with a purity better than 97%, were commercial products or kindly provided by Beecham Pharmaceuticals, U.K. or Eli Lilly and Company, U.S.A. The 4-imidazolidinones derived from ampicillin and acetone (i.e. hetacillin) or acetaldehyde were prepared as described by Hardcastle et al. (1966) and Johnson and Panetta (1965), respectively. Buffer substances and all other chemicals or solvents used were of reagent grade. Formaldehyde was used in the form of a 37% aqueous solution.

Apparatus

High-performance liquid chromatrography (HPLC) was done with a Spectraphysics Model 3500B instrument equipped with a variable-wavelength UV detector (8-μl 1 cm flow cells) and a 10-μl loop injection valve. The column used, 250 × 4 mm, was packed with LiChrosorb RP-8 (7 μm particles) (E. Merck, F.R.G.). Visible spectral measurements were performed with a Shimadzu UV-190 spectrophotometer, using 1 cm cuvettes. Readings of pH were carried out on a Radiometer Type PHM 26 meter at the temperature of study.

Kinetic measurements

All rate studies were performed in aqueous buffer solutions at 37.0 ± 0.2°C. The buffers used were acetate, phosphate and borate solutions. The total buffer concentration was 0.05 M except in experiments where buffer effects were studied specifically. A constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride.

The rates of reaction of ampicillin with various aldehydes and ketones were determined by using a reversed-phase HPLC procedure which enabled separation and simultaneous quantitation of ampicillin and the corresponding imidazolidinones. The mobile phases used were 0.02 M phosphate pH 7.0 containing methanol in a concentration of 25–40% v/v, depending upon the carbonyl compound, or containing 10% v/v acetonitrile in the case of reactions with formaldehyde (and reaction of amoxycillin with acetaldehyde). The elution was done at ambient temperature at a rate of 1.2 ml · min^{-1} and the column effluent was monitored at 220 nm. Quantitation was done from measurement of peak heights.

The reactions were initiated by adding 100 μl of an aqueous stock solution of ampicillin sodium to 10 ml of pre-heated buffer solution containing the carbonyl compound. The initial concentration of ampicillin was about 10^{-4} M whereas the initial concentration of the carbonyl compounds varied from 5×10^{-4} to 0.5 M. The reaction solutions were kept at 37°C and aliquots were removed at suitable intervals and chromatographed. Rate and equilibrium constants were calculated as described below.

The reaction of ampicillin with formaldehyde and acetaldehyde was also performed under conditions in which ampicillin was used in large excess (0.02–0.003 M). The initial aldehyde concentration was about 7×10^{-4} M and the reaction progress was followed by monitoring the decrease in aldehyde concentration using the colorimetric method previously described (Johansen et al., 1983). Pseudo-first-order rate constants were calculated from the slopes of linear plots of log $(A_t - A_\infty)$ against time, where A_t and A_∞ are the absorbance readings at time t and at infinity, respectively.

Fig. 1.

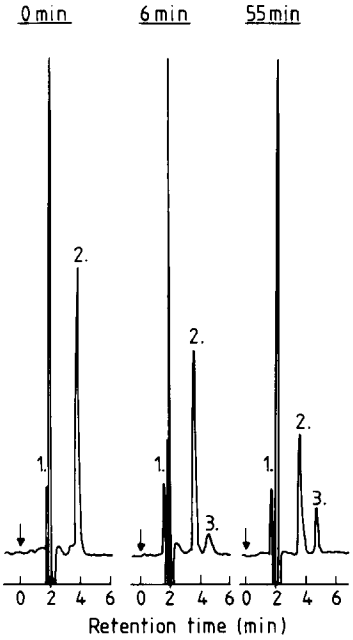


Fig. 2.

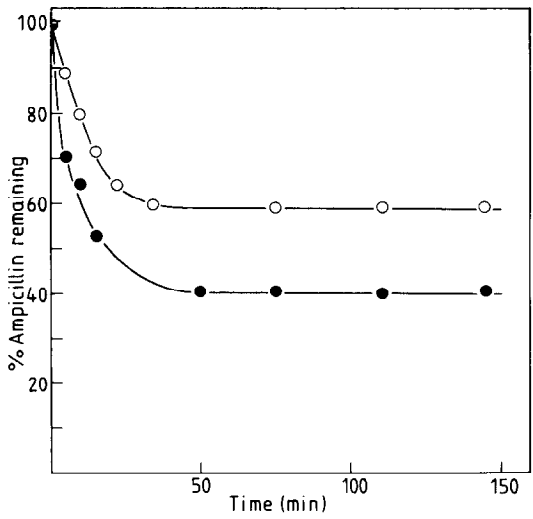


Fig. 1. High-performance liquid chromatographic traces of the formation of the 4-imidazolidinone derivative from reaction of ampicillin sodium (10^{-4} M) with propionaldehyde (1.6×10^{-3}) in 0.05 M phosphate buffer solution of pH 7.45 at 37°C. A 10 μl sample of the solution was chromatographed at the times indicated. HPLC mobile phase: methanol–0.02 M phosphate, pH 7.0 (35 : 65 v/v). Peak identities: 1, solvent front; 2, ampicillin; 3, the 4-imidazolidinone derivative.

Fig. 2. Time-courses for ampicillin in reaction with propionaldehyde (8.0×10^{-4} M (○); 1.6×10^{-3} M (●)) in 0.05 M phosphate buffer solution of pH 7.45 at 37°C. The initial concentration of ampicillin was 10^{-4} M.

Results and Discussion

The kinetics of reaction of various aldehydes or ketones with ampicillin was studied in aqueous buffer solutions at 37°C by measuring the disappearance of ampicillin using HPLC. Most kinetic runs were conducted under pseudo-first-order conditions in which the carbonyl compounds were used in large excess over ampicillin. As revealed by HPLC (Fig. 1) the loss of ampicillin was accompanied by the formation of products possessing a retention time slightly greater than that of ampicillin. In case of reaction with acetone and acetaldehyde these reaction products were proved to be the corresponding 4-imidazolidinones on the basis of comparison of the retention data with those of authentic 4-imidazolidinones. It has previously been shown that ampicillin readily condenses with various aldehydes and ketones in great excess to give the corresponding 4-imidazolidinones (Panetta and Johnson, 1965).

Under the experimental conditions used all reactions proceeded to an equilibrium. A typical example of the time course for ampicillin is shown in Fig. 2. With the carbonyl compounds being in large excess the rate of ampicillin disappearance followed strict reversible first-order kinetics and pseudo-first-order rate contants, k_{obs}, were determined from the slope of linear plots of log $(A_t - A_{eq})$ against time, where A_t and A_{eq} are the concentrations of ampicillin at time t and at equilibrium, respectively. In Table 1 are listed values of k_{obs} and extent of reaction at given initial concentrations of the carbonyl compounds. The values of k_{obs} were found to be linearly related to the initial aldehyde or ketone concentration, $[C]_0$, and plots of

TABLE 1

OBSERVED PSEUDO-FIRST-ORDER RATE CONSTANTS (k_{obs}) AND EXTENT OF REACTION FOR THE REVERSIBLE REACTION OF AMPICILLIN WITH VARIOUS CARBONYL COMPOUNDS IN AQUEOUS SOLUTION OF pH 7.45 (37°C; $\mu = 0.5$) [a]

Carbonyl compounds	Conc. (M)	k_{obs} (min^{-1})	% Ampicillin at equilibrium
Formaldehyde	10^{-3}	0.0023	~ 3
Acetaldehyde	10^{-3}	0.13	60
Propionaldehyde	10^{-3}	0.096	54
Butyraldehyde	10^{-3}	0.13	60
Isobutyraldehyde	10^{-3}	0.028	77
Isovaleraldehyde	10^{-3}	0.248	65
Benzaldehyde	10^{-3}	0.0056	92
Acetone	10^{-1}	0.040	93
	10^{-3}	0.037 [b]	99.9 [b]
Pyruvic acid	10^{-1}	0.0076	9
	10^{-3}	0.0008 [b]	91 [b]

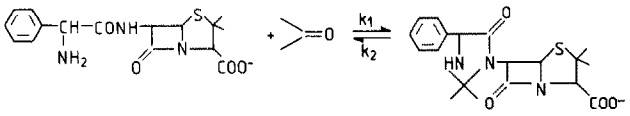
[a] The initial concentration of ampicillin sodium was 10^{-4} M so that pseudo-first-order conditions were maintained during the kinetic runs.

[b] Calculated data.

k_{obs} against $[C]_0$ had a positive intercept when extrapolated to $[C]_0 = 0$ (Fig. 3). Thus, it can be concluded that the reactions taking place are as depicted in Scheme 2 and that the following rate expression is valid:

$$k_{obs} = k_2 + k_1[C]_0 \tag{1}$$

where k_1 is a second-order rate constant for 4-imidazolidinone formation and k_2 is a pseudo-first-order rate constant for hydrolysis of the 4-imidazolidinones.



To determine the rate constants k_1 and k_2 and hence the equilibrium constant $K(= k_1/k_2)$, plots of k_{obs} against $[C]_0$ according to Eqn. 1 was used. Since the intercept values from such plots in some cases were not determined precisely, the following method of calculation was also used.

The equilibrium constant was derived from the following equation:

$$K = \frac{[A]_0 - [A]_{eq}}{[A]_{eq} \times [C]_0} \tag{2}$$

where $[A]_0$ is the initial ampicillin concentration. For convenience peak heights of ampicillin were used instead of concentrations. Substituting k_1 with $K \times k_2$ in Eqn. 1 affords:

$$k_2 = \frac{k_{obs}}{1 + K[C]_0} \tag{3}$$

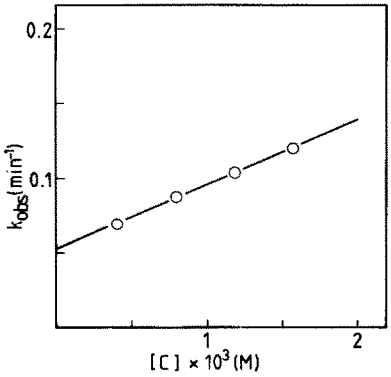


Fig. 3. Plot of rate data for the reaction of ampicillin with propionaldehyde at pH 7.45 and 37°C according to Eqn. 1.

from which values of k_2 were obtained. Finally, k_1 was determined according to:

$$k_1 = K \times k_2 \quad (4)$$

The values of k_1, k_2 and K thus obtained for reactions at physiological pH and temperature are listed in Table 2.

The reactions of ampicillin with formaldehyde and acetaldehyde were also followed under conditions where ampicillin was in excess of the aldehydes. In this case the reaction progress was monitored by determining the decrease in aldehyde concentration using the spectrophotometric method previously described (Johansen et al., 1983). The rate and equilibrium constants thereby obtained agreed within $\pm 10\%$ with those given in Table 2. For hetacillin the rate constant (k_2) for its hydrolysis at pH 7.45 and 37°C was also determined directly in a separate run using a 10^{-4} M solution of the compound. A k_2-value of 0.042 min^{-1} was obtained which is in good agreement with the value (0.037 min^{-1}) derived as described above.

Amoxycillin and cephalexin behaved towards acetaldehyde as ampicillin. At pH 7.45 the following data were obtained: $K = 9.0 \times 10^2$ M^{-1}, $k_1 = 55$ $M^{-1} \cdot min^{-1}$ and $k_2 = 6.1 \times 10^{-2}$ min^{-1} (amoxycillin); $K = 9.7 \times 10^2$ M^{-1}, $k_1 = 44$ $M^{-1} \cdot min^{-1}$ and $k_2 = 4.6 \times 10^{-2}$ min^{-1} (cephalexin).

The results obtained show that the rate of 4-imidazolidinone formation is depressed by increasing steric effect within the carbonyl compounds. The ketones are much less reactive than the aldehydes. In considering the k_1-value for formaldehyde it should be recognized that this aldehyde is strongly hydrated in aqueous solution. Thus, its hydration equilibrium constant is 10^3 M^{-1} at 25°C whereas that of the other aliphatic aldehydes is only 0.4–0.9 M^{-1} (Gruen and McTigue, 1963). This was not taken into account in the calculations. Considering the stability of the imidazolidinones of ampicillin (i.e. the k_2-values) it does not vary much for the compounds derived from the aliphatic aldehydes and acetone, the half-lives being 4–31 min at pH 7.45 and 37°C. An exception is the compound derived from formaldehyde, its half-life being 29 h at neutral pH. The relatively small influence of

TABLE 2

RATE OF EQUILIBRIUM CONSTANTS FOR THE REACTION OF AMPICILLIN WITH VARIOUS ALDEHYDES AND KETONES IN AQUEOUS SOLUTION AT pH 7.45 (37°C; $\mu = 0.5$)

Carbonyl compound	k_1 ($M^{-1} \cdot min^{-1}$)	k_2 (min^{-1})	K (M^{-1})
Formaldehyde	2.3	$\sim 4 \times 10^{-4}$	$\sim 6 \times 10^3$
Acetaldehyde	51	7.7×10^{-2}	6.6×10^2
Propionaldehyde	44	5.2×10^{-2}	8.5×10^2
Butyraldehyde	53	8.0×10^{-2}	6.7×10^2
Isobutyraldehyde	6.0	2.2×10^{-2}	3.0×10^2
Isovaleraldehyde	83	1.6×10^{-1}	5.3×10^2
Benzaldehyde	0.41	5.2×10^{-3}	8.2×10^1
Acetone	0.030	3.7×10^{-2}	0.80
Pyruvic acid	0.069	7.1×10^{-4}	9.4×10^1

TABLE 3

RATE AND EQUILIBRIUM CONSTANTS FOR THE REACTION OF AMPICILLIN WITH ACETALDEHYDE IN AQUEOUS SOLUTION AT VARIOUS pH VALUES (37°C; $\mu = 0.5$)

pH	k_1 ($M^{-1} \cdot min^{-1}$)	k_2 (min^{-1})	K (M^{-1})
6.00	11	0.08	130
6.48	16	0.07	220
6.98	29	0.07	420
7.45	51	0.07	660
7.70	58	0.07	780
8.43	30	0.04	775
8.90	17	0.03	595

the carbonyl component on the reactivity of the ampicillin imidazolidinones is somewhat surprising in view of the substantial effect observed for the related oxazolidinones (Johansen and Bundgaard, 1983).

The reaction of ampicillin with acetaldehyde was studied as a function of pH within the range pH 6–9. The rate and equilibrium constants obtained at the various pH values are shown in Table 3. Whereas the k_2-values do not vary much within this pH range which coincides with the stability behaviour of other 4-imidazolidinones (Klixbüll and Bundgaard, 1984) the k_1 constants (and hence also the equilibrium constants) show a bell-shaped dependence on pH (Fig. 4). This shape indicates the involvement of a kinetically significant intermediate in the reaction pathway and a change of the rate-determining step in the overall reaction with pH. A most plausible intermediate is a Schiff base formed between the carbonyl compound and the free

Fig. 4.

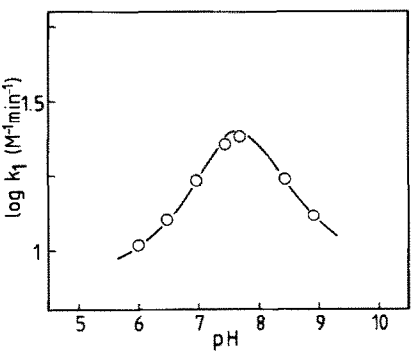


Fig. 4. The influence of pH upon the second-order rate constant (k_1) for the reaction of ampicillin with acetaldehyde in aqueous solution in 37°C.

Fig. 5.

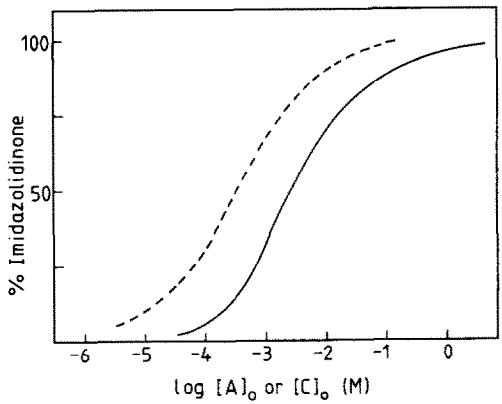


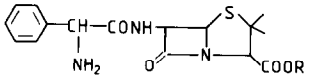
Fig. 5. The percentage equilibrium concentration of imidazolidinone derivative formed by reaction of ampicillin with acetaldehyde (————) or formaldehyde (------) at pH 7.45 and 37°C as calculated from the equilibrium constants given in Table 2.

amino group of ampicillin and in fact, Durbin and Rydon (1970) have previously, using an NMR spectroscopic method, provided evidence for the existence of a Schiff base intermediate in the interconversion between ampicillin, acetone and hetacillin in aqueous solution.

Fujiwara et al. (1982a, b and c) have recently reported that the degradation of ampicillin in neutral and basic solutions is markedly inhibited in the presence of benzaldehyde or furfural. The effect was ascribed to the formation of an adduct between ampicillin and the aldehydes, its structure being supposed to be a Schiff base. The equilibrium formation constant for the adduct of benzaldehyde was 48 and 77 M^{-1} at pH 7 and 8, respectively (35°C). On the basis of the present study it appears more likely that the adduct concerned is a 4-imidazolidinone rather than a Schiff base. However, as noted above, these structures are likely to be in equilibrium in aqueous solution.

Two ampicillin prodrugs, pivampicillin (IIIa) and bacampicillin (IIIb), release formaldehyde and acetaldehyde, respectively, upon their bioconversion to the parent drug. When the hydrolysis of these compounds (8×10^{-4} M) in human plasma solutions was followed by quantitating the aldehyde released, the yield of aldehyde only amounted to about 50% (formaldehyde) and about 70% (acetaldehyde) (Johansen et al., 1983). It was suggested that this decreased yield is due to a secondary reaction of the aldehydes with the ampicillin side-chain with formation of a 4-imidazolidinone derivative (Johansen et al., 1983). The results of the present study provide strong support for this explanation. From the experimentally determined equilibrium constants for the reaction of ampicillin with the aldehydes the concentration of the imidazolidinone derivative at equilibrium can easily be calculated at various initial (and equal) concentrations of ampicillin and the aldehydes. The results of such calculations are shown in Fig. 5. At initial concentrations of ampicillin and the aldehydes of 8×10^{-4} M it can be seen that at equilibrium the corresponding 4-imidazolidinone derivative is present in amounts corresponding to 28% (acetaldehyde) and 65% (formaldehyde). This corresponds to a decreased aldehyde concentration of 72% (acetaldehyde) and 35% (formaldehyde).

Several metabolites of drugs are formed by non-enzymatic condensations with biogenic carbonyl compounds such as acetaldehyde, formaldehyde, acetone or pyruvic acid (O'Donnell, 1982). In considering the possible in vivo formation of 4-imidazolidinones from reaction of ampicillin with biogenic aldehydes and ketones the magnitude of the equilibrium constants and the k_2 rate constants is of importance. Thus, it can be estimated that no significant imidazolidinone formation with acetone, acetaldehyde or pyruvic acid is likely to occur due either to the ready reversibility of the reaction or to a low equilibrium constant. An imidazolidinone



IIIa $R = CH_2OOC(CH_3)_3$

IIIb $R = CHOCOC_2H_5$ (CH_3, O)

formation may, however, possibly occur in vivo by reaction with formaldehyde. The normal concentration of endogenous releasable formaldehyde in human blood is about 2×10^{-5} M (Trézl et al., 1983). At a similar ampicillin concentration the calculations shown in Fig. 5 reveal that at reaction equilibrium the imidazolidinone derivative which has a half-life of 29 h at physiological conditions of pH and temperature would be present in an amount corresponding to 15%.

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