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Research Papers

A DIFFERENTIAL KINETIC METHOD FOR THE DETERMINATION OF MECILLINAM IN THE PRESENCE OF ITS HYDROLYSIS AND EPIMERIZATION PRODUCTS

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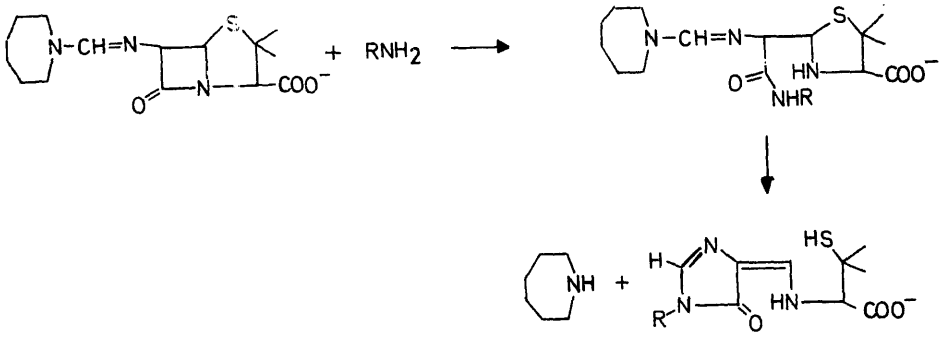
SUMMARY

A differential kinetic method is described for the selective determination of mecillinam in the presence of its degradation products including (6S)-mecillinam formed by a C_6-epimerization. The method involves spectrophotometric measurement at 330 nm of a 4-aminomethyleneimidazol-5(4H)-one derivative, formed by reaction of mecillinam with an aqueous glycine buffer at 35°C, and is based on the different rates of reaction of mecillinam and its 6-epimer with the glycine reagent. The procedure makes use of the method of proportional equations and it permits simultaneous determination of mecillinam and its 6-epimer. The accuracy and precision of the procedure were evaluated and its applicability for assessing the stability of mecillinam was demonstrated. C_6-epimerization to yield (6S)-mecillinam was shown to account for 30% of the total degradation of mecillinam in aqueous solution at pH 9.9 and 35°C.

INTRODUCTION

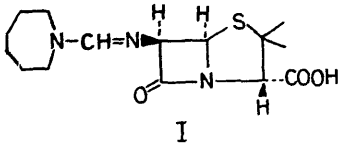
The reaction of primary amines (e.g. glycine) with mecillinam, (6R)-6-[(hexahydro-1H-azepin-1-yl)methyleneamino]penicillanic acid (I), to produce a strongly ultraviolet-absorbing (λ_{max} 330 nm) 4-aminomethyleneimidazol-5(4H)-one derivative, formed by an intramolecular cyclization of the initially produced N-penicilloylamine (Scheme 1), (Bundgaard, 1977) has been utilized as the basis of a sensitive spectrophotometric method for the quantitative determination of this new β-lactam antibiotic (Larsen and Bundgaard, 1977a). Since both in intact β-lactam ring and an amidine group at position 6 is necessary for the method and since hydrolysis of the drug in aqueous solution over a broad pH range results in destruction of one or both of these moieties (Larsen and Bundgaard, 1977b; Baltzer et al., 1979), no interference in the method by degradation products should be expected. Recently, however, Baltzer et al. (1979) have shown that in



Scheme 1

neutral and basic aqueous solutions mecillinam undergoes C_6-epimerization to produce (6S)-mecillinam (II) in addition to hydrolysis in the β-lactam ring and in the amidino side-chain. The 6-epimer of mecillinam interfered strongly in the spectrophotometric assay, thus restricting the general use of this method as a stability-indicating assay.

In the present paper a differential kinetic method is described by which the interference of the 6-epimer in the spectrophotometric assay can be eliminated. In addition, the method permits a simultaneous quantitation of both epimers. The utility of the kinetic method for assessing the stability of mecillinam is demonstrated and some rate data for the epimerization process in alkaline solution are included in the paper.



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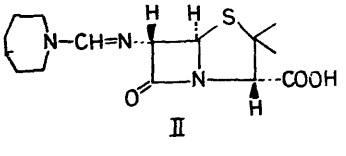

II

MATERIALS AND METHODS

Apparatus

A Perkin-Elmer Model 121 spectrophotometer and a Radiometer Model PHM 26 pH meter were used for the measurements.

Materials and reagents

Mecillinam with a potency of 99.4% and samples of 6-epimecillinam (6S)-6[(hexahydro-1H-azepin-1-yl)methyleneamino]penicillanic acid) and 6-formamidopenicillanic acid were kindly supplied by Dr. F. Lund, Leo Pharmaceuticals, Ballerup, Denmark, All other chemicals used were of analytical grade.

Glycine reagent. This is a 1.0 M glycine solution of pH 10.2 ± 0.1. It was prepared by

dissolving 75 g of glycine in 340 ml of 2 M sodium hydroxide and diluting with water to 1000 ml.

Analytical procedure

Prepare a solution in distilled water of mecillinam at a concentration of about 5×10^{-4} M ($\sim 160\ \mu g\ ml^{-1}$). Pipette 5.00 ml of the glycine reagent into each of two test tubes and place them in a water bath at 35°C. Add 500 µl of the test solution to each of the tubes, stopper them and allow the solutions to stand at 35°C. After exactly 13 min, one reaction solution is removed from the water bath and is quenched by the addition of 500 µl of 5 M hydrochloric acid and cooling to room temperature. After standing for exactly 30 min, the remaining solution is similarly treated. Determine absorbance values at 330 nm (1-cm cells) for each mecillinam solution within 5 min using distilled water as reference (the reagents possess no absorption at 330 nm). Calculation of the concentration of mecillinam and its 6-epimer in the test sample is done as described below using Eqn. 3.

Determination of degradation course of mecillinam in alkaline solution

The degradation of mecillinam was studied in a 0.05 M borate buffer solution ($\mu = 0.5$ with potassium chloride) of pH 9.90 at 35°C. An accurately weighed quantity of mecillinam (about 10 mg) was dissolved in 50 ml of the borate buffer pre-equilibrated at 35°C. At appropriate times 500 µl aliquots were withdrawn and analyzed for mecillinam and (6S)-mecillinam by the differential reaction rate method described above.

The degradation of (6S)-mecillinam in the borate buffer solution was studied in a similar way, the initial concentration of the compound being 10^{-3} M.

RESULTS AND DISCUSSION

When subjecting the 6-epimer of mecillinam ((6S)-mecillinam) to the spectrophotometric assay previously described for mecillinam (Larsen and Bundgaard, 1977a) it was observed that the compound reacted somewhat slower with the glycine reagent. In the original method the glycine reagent (1 M glycine of pH 10.2) contained 2×10^{-4} M mercury (II) chloride and a reaction temperature of 60°C was used. Under these conditions the reaction of mecillinam with the glycine reagent to produce a 4-aminoethyleneimidazol-5(4H)-one derivative (Scheme 1) is complete after 30 min. Reaction of the 6-epimer was found to be complete after about 45 min, giving a molar absorptivity in this assay of 70% of the absorptivity of mecillinam.

It has now been observed that when mercury (II) chloride is omitted from the glycine solution the rates of production of the 4-aminomethyleneimidazol-5(4H)-one derivative from the reaction of glycine with the two 6-epimers differ much more than in the presence of the mercuric salt. This difference in reaction rate has been utilized to design a kinetic procedure permitting a selective determination of mecillinam or, when both epimers are present in admixture, a simultaneous quantitation of the components.

Fig. 1 shows the time-courses for the imidazolone formation from the mecillinam epi-

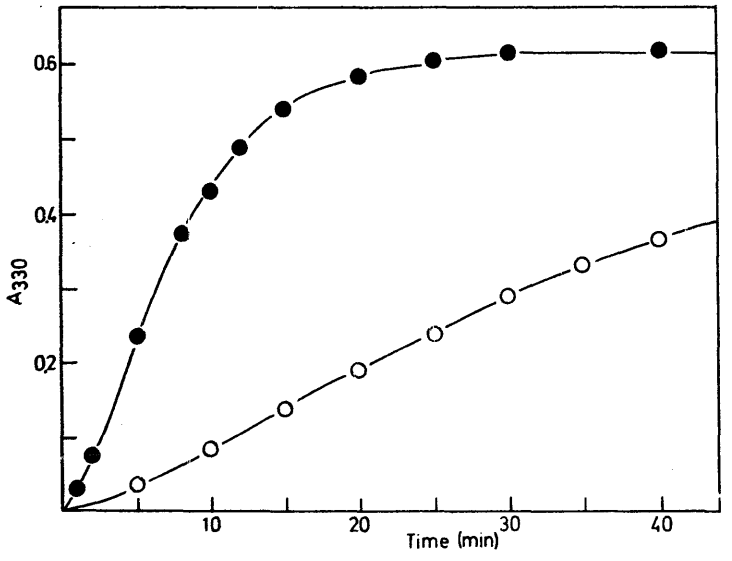

Fig. 1. Absorbance–time curves for treatment of mecillinam (•) and (6S)-mecillinam (○) with the glycine reagent at 35°C. The concentrations of the compounds in the final reaction solutions were $4.^{1}$ 10^{-5} M.

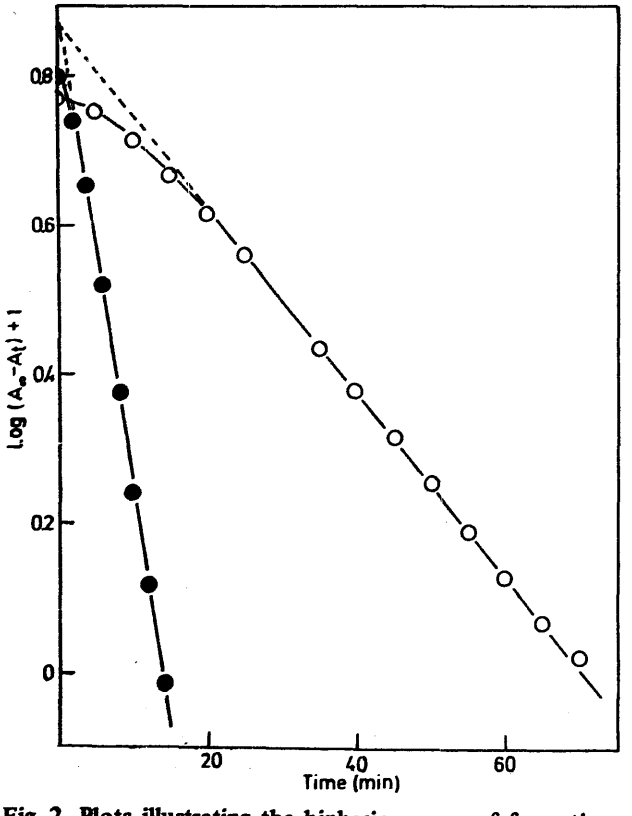

Fig. 2. Plots illustrating the biphasic course of formation of imidazolone derivative in the reaction solutions of mecillinam (•) and (6S)-mecillinam (○) from Fig. 1.

mers in the glycine solution at 35°C. It is readily apparent from Fig. 1 that the (6S)-epimer reacts more slowly than the (6R)-epimer and also, that the reactions show a biphasic course passing through an intermediate. In Fig. 2 the logarithm of the function ($A_\infty - A_t$) has been plotted against time, where A_∞ and A_t represent the absorbances at infinity and at time t, respectively. The linear portions of the curves in Fig. 2 apparently represent a slow reaction following an initial faster reaction. The faster reaction is due to reaction of glycine with the β-lactam moiety in the mecillinam molecule to produce an N-penicilloylglycine derivative (see Scheme 1) and the slower reaction step is due to an intramolecular rearrangement of this derivative to yield the imidazolone product (Bundgaard, 1977). From the slopes of the straight-line portions of the plots in Fig. 2 apparent first-order rate constants representing the slower step in the imidazolone formation were calculated to be 0.16 min^{-1} (mecillinam) and 0.029 min^{-1} ((6S)-mecillinam). Using the procedure previously described (Bundgaard, 1977) pseudo-first-order rate constants of 0.52 min^{-1} (for mecillinam) and 0.067 min^{-1} (for (6S)-mecillinam) were determined for the initial faster reaction step.

Among the most commonly used differential kinetic methods for analyzing closely related compounds in admixture, the method of proportional equations of the double-point method (Garmon and Reilley, 1962) was employed for the present purpose. This method is applicable for both simple and complex reactions (such as the present ones) which exhibit a constant fractional life (Mark and Rechnitz, 1968). The method consists of making measurements at two different times, substituting these values into a pair of simultaneous equations, and solving for the concentrations of both components. The equations are:

$$P_t = K_A[A]_0 + K_B[B]_0 \tag{1}$$

$$P_{t'} = K'_A[A]_0 + K'_B[B]_0 \tag{2}$$

where P_t and $P_{t'}$ are parameters proportional to concentration (in this case absorbance) measured at times t and t'. The initial concentrations of the components are expressed as $[A]_0$ and $[B]_0$, and K_A, K_B, K'_A and K'_B are constants corresponding to the slopes of plots of P_t and $P_{t'}$ vs concentrations at times t and t', respectively.

Solvings Eqns. 1 and 2 simultaneously for $[A]_0$ and $[B]_0$, respectively, the following expressions are obtained:

$$[A]_0 = \frac{P_t - P_{t'}(K_B/K'_B)}{K_A - K_B(K'_A/K'_B)} \tag{3}$$

$$[B]_0 = \frac{P_t - P_{t'}(K_A/K'_A)}{K_B - K_A(K'_B/K'_A)} \tag{4}$$

For the analysis of mixtures of the two mecillinam 6-epimers, the longer reaction time (t') was chosen to be 30 min at which time the reaction of (6R)-mecillinam is essentially completed and the (6S)-mecillinam reaction is approximately 45% completed. Once this long time was selected, a shorter reaction time, t, of 13 min was found to be optimum using the graphical approach to time selection described by Garmon and Reilley (1962).

As seen from Fig. 3 the absorbances produced upon reaction of the epimeric penicil-

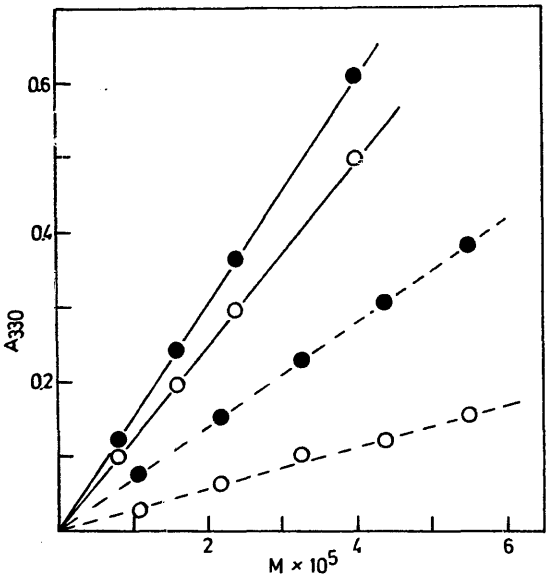

Fig. 3. Plots of absorbance at 330 nm vs mecillinam (———) and (6S)-mecillinam (- - - - -) concentration in the reaction solutions after reaction times of 13 min (○) and 30 min (●).

lanic acids after these reaction times are directly proportional to the initial concentrations. The values of the proportionality constants calculated from these data are listed in Table 1.

Accuracy and precision of the kinetic method

In order to obtain accurate and reproducible results with the kinetic method it is necessary to rigidly control reaction conditions such as temperature and reaction times. With respect to the latter, addition of hydrochloric acid in the amount described was found to depress the reaction progress so the absorbance remained stable for at least 5 min at room temperature.

To evaluate the accuracy and precision of the method binary mixtures of the two epimers in different proportions were prepared and analyzed. The results obtained are given

TABLE 1

EXPERIMENTAL PROPORTIONALITY CONSTANTS OF MECILLINAM AND (6S)-MECILLINAM FOR THE REACTION TIMES t = 13 MIN (K) AND t' = 30 MIN (K') [a]

Compound	K (M^{-1})	K' (M^{-1})
Mecillinam	12.5×10^3	15.2×10^3
(6S)-Mecillinam	2.8×10^3	7.0×10^3

[a] The K-values were determined from the slopes of linear plots of absorbance at the appropriate reaction time vs molar concentration of compound in the reaction solution.

TABLE 2

ANALYSIS OF MIXTURES OF MECILLINAM AND (6S)-MECILLINAM

Mecillinam ($\mu g\ ml^{-1}$)		(6S)-Mecillinam ($\mu g\ ml^{-1}$)	
Taken	Found	Taken	Found
0	0	200.0	201.6 ± 3.8 [a]
10.0	8.8	190.0	191.0
20.0	21.0	180.0	180.8
50.0	50.8	150.0	148.1
100.0	98.4 ± 2.5 [a]	100.0	100.8 ± 3.2 [a]
150.0	148.1	50.0	51.4
180.0	180.8	20.0	19.2
190.0	191.0	10.0	8.2
200.0	199.1 ± 3.2 [a]	0	0

[a] Mean values ± S.D. of 8 analyses. All other results are averages of two determinations.

in Table 2. The minimum concentrations of the components that can be analyzed in mixtures within tolerable limits of error are about 5%.

Application to assessing stability of mecillinam

Like the originally described equilibrium method (Larsen and Bundgaard, 1977a), the kinetic assay requires both an intact β-lactam ring and an amidino group at position 6 of the mecillinam molecule. Degradation of the drug by hydrolysis in aqueous solutions over the pH range 0–13 results in destruction of the one or both of these moieties (Larsen and Bundgaard, 1977b; Baltzer et al., 1979) and thus, no interference in the method by hydrolytic degradation products should be expected. It was directly proved that 6-formamidopenicillanic acid, a major degradation product at pH 6–10 (Larsen and Bundgaard, 1977b), does not interfere in the method nor do other penicillins such as benzylpenicillin.

The applicability of the kinetic method for assessing the stability of mecillinam was investigated with a 0.05 M borate buffer solution (pH 9.90) as test solution since 6-epimerization of mecillinam has been reported to occur in basic aqueous solution concurrently with hydrolytic degradations (Baltzer et al., 1979). As described in the experimental section, aliquots of the reaction solution were periodically assayed for remaining mecillinam and formed (6S)-mecillinam according to the kinetic procedure. In Fig. 4 are shown the time-courses for mecillinam and its 6-epimer. The latter is seen to reach a maximal concentration of 18% during the reaction and its formation is followed by a slower decomposition. This figure is in good agreement with the results of Baltzer et al. (1979). On basis of TLC and NMR measurements these investigators found (6S)-mecillinam to be formed in amounts of about 20% upon degradation of mecillinam at pH 10 and 37°C. The inset of Fig. 4 is a semi-logarithmic plot of the concentration–time data for mecillinam and it demonstrates that the overall loss of the drug in the borate solution follows strict first-order kinetics for several half-lives when monitored by the kinetic method.

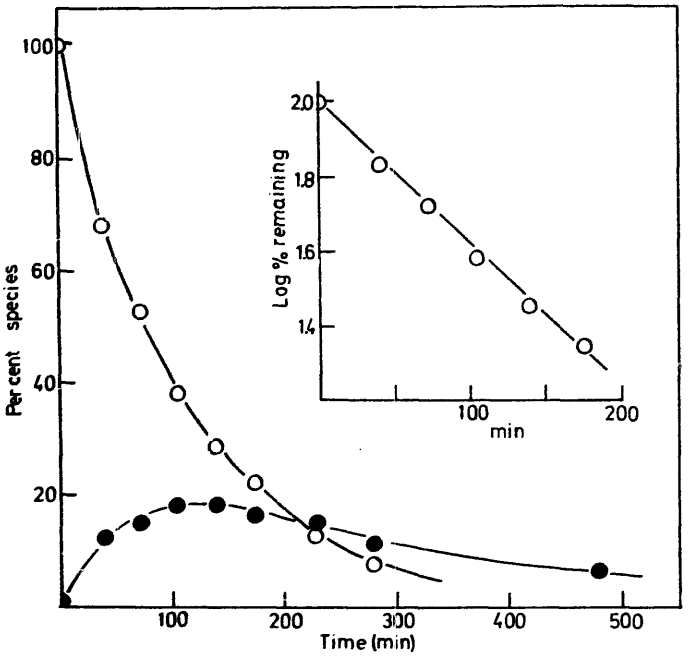

Fig. 4. Time-courses for mecillinam (○) and (6S)-mecillinam (●) in a 0.05 M borate buffer solution (μ = 0.5) at pH 9.90 and 35°C. The concentrations at various times, expressed as percent in relation to the initial mecillinam concentration, were determined by the described kinetic procedure. The inset is a semi-logarithmic plot of the concentration–time data for mecillinam.

The overall reactions occurring in the borate buffer solutions may be described by the following scheme:

$$\text{(6R)-mecillinam} \xrightarrow{k_1} \text{(6S)-mecillinam} \xrightarrow{k_1} \text{hydrolysis products}$$
$$\downarrow k_3$$
$$\text{hydrolysis products}$$

The pseudo-first-order rate constants (k_1–k_3) associated with the various reactions in this scheme were determined in the following manner.

From the slope of the semi-logarithmic plot of Fig. 4 a pseudo-first-order rate constant (k_{obs}) for the overall degradation of mecillinam of 0.0092 min^{-1} was calculated. The rate constant k_2 for the hydrolysis of (6S)-mecillinam was obtained from a separate run with the compound in the borate solution and a value of 0.0036 min^{-1} was found. In the experiment described by the data of Fig. 4 (6S)-mecillinam has a time-dependence given by the following expression:

$$[6S]_t = \frac{k_1}{k_{obs} - k_2} [6R]_0 (e^{-k_2 t} - e^{-k_{obs} t}) \qquad (5)$$

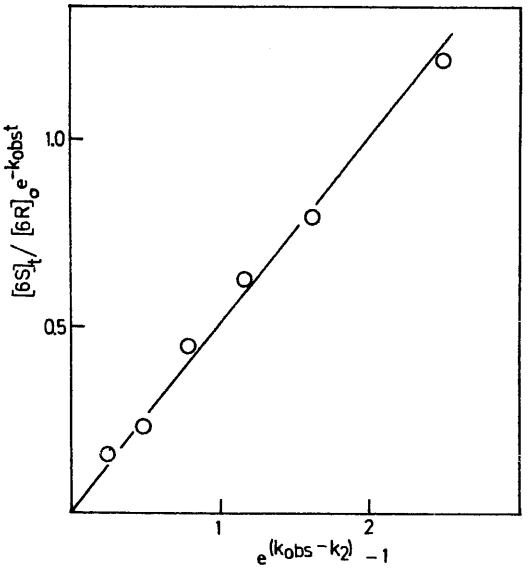

Fig. 5. Derivation of the rate constant k_1 by plotting the data of Fig. 4 according to Eqn. 6.

where $[6R]_0$ represents the initial concentration of mecillinam and $[6S]_t$ is the concentration of (6S)-mecillinam at time t. Rearrangement of Eqn. 5 affords Eqn. 6:

$$\frac{[6S]_t}{[6R]_0\, e^{-k_{obs}t}} = \frac{k_1}{k_{obs} - k_2}\,[e^{(k_{obs}-k_2)t} - 1] \qquad (6)$$

Eqn. 6 indicates that a plot of the left side of the equation against $(e^{(k_{obs}-k_2)t} - 1)$ should yield a straight line passing through the origin at zero time. Such a plot of the data in Fig. 4 is shown in Fig. 5. From the slope of the resulting straight line the ratio $k_1/(k_{obs} - k_2)$ was determined to be 0.51. Using the known values of k_{obs} and k_2 a value of 0.0028 min^{-1} for k_1 was obtained. Finally, the rate constant, k_3, was calculated to be 0.0064 min^{-1} using the identity $k_{obs} = k_1 + k_3$.

From the ratio $k_1 : k_{obs}$ the relative contribution of C_6-epimerization to the overall degradation of mecillinam at the given reaction conditions can be calculated to be 30%. According to Baltzer et al. (1979) the epimerization is reversible, the equilibrium, however, being strongly in favour of the 6R-epimer. Therefore, in the reaction scheme described above it may be justified to neglect a reversible reaction. The occurrence of a reversible epimerization could, however, be confirmed in the rate experiment starting with (6S)-mecillinam. After 2 h when about 35% of the compound had degraded, 3 mole · % of (6R)-mecillinam appeared as determined by the kinetic method.

In summary, it appears that the differential kinetic method described permits a selective determination of mecillinam in the presence of its various degradation products including the 6S-epimer and at the same time makes it possible to quantitate this epimeric product.

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