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First derivative spectrophotometric, TLC-densitometric, and HPLC determination of acebutolol HCL in presence of its acid-induced degradation product

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Abstract

Three methods are presented for the determination of acebutolol HCl in presence of its acid-induced degradation product. The first method was based on measurement of the first derivative amplitude of acebutolol HCl at 266.6 nm. The second method was based on separation of acebutolol HCl from its acid-induced degradation product followed by densitometric measurement of the spots at 230 nm. The separation was carried out on silica gel 60 F_{254}, using ethanol–glacial acetic acid (4:1, v/v) as mobile phase. Second order polynomial equation was used for the regression line. The third method was based on high performance liquid chromatographic (HPLC) separation of acebutolol HCl from its acid-induced degradation product on a reversed phase, ODS column using a mobile phase of methanol–water (55:45, v/v) with UV detection at 240 nm. The first derivative spectrophotometric method was utilized to investigate the kinetics of the acid degradation process at different temperatures. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Acebutolol HCl; First-derivative spectrophotometry; HPLC; Kinetics of degradation; TLC-densitometry

1. Introduction

Acebutolol HCl (**I**), 3′-acetyl-4′-[2-hydroxy-3-(Isopropylamino) propoxy] butyranilide hydrochloride, is a beta-adrenergic antagonist used as antihypertensive, antianginal and antiarrhythmic drug. The official method for determination of (**I**) is potentiometric acid–base titration [1], and HPLC [2] using octadecyl silane column and methanol–aqueous solution of dodecyl sodium sulfate–glacial acetic acid (675:325:20) as mobile phase. Various spectrophotometric methods have been reported for the determination of (**I**) in pharmaceutical formulation using different

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reagents including iodine in presence of isonicotinic acid hydrazide [3] or Wool Fast blue BL [4], Folin-ciocalteu [5], sodium nitrite with HCl and chromotropic acid or phloroglucinol or ethyl acetoacetate [6], metol and potassium permanganate [7], carbon disulfide and copper (II) ions [8], chloranilic acid, 2,6-dichlorophenolindophenol or 2,3-dichloro-5,6-dicyano-p-benzoquinone [9], choranil,2-5-dichlorobenzoquinone or 2,6-dibromobenzoquinone chlorimine [10], bromothymol blue [11] and 3-methylbenzothiazolin-2-one hydrazone hydrochloride in presence of ceric ammonium sulfate[12] or ferric chloride [13]. The chromatographic techniques of analyses, HPLC using chiral separation [14] or dabsyl derivatives [15] and GC [16] have been employed. Many methods have been applied for the determination of (I) in a mixture with hydrochlorothiazide using HPLC [17], with atenolol and hydrochlorothiazide by spectrometric analysis [18], and with nifedipine using UV-derivative spectroscopy, capillary GC and HPLC [19].

The International Conference on Harmonization (ICH) guideline entitled "stability testing of new drug substances and products" requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance [20]. It suggests that the degradation products that are formed under a variety of conditions should be identified and degradation pathways established. Susceptibility to hydrolysis under acidic conditions is one of the required tests. No method has been reported in the literature for the determination of (I) in presence of its acid–induced degradation products. Therefore, it was thought necessary to study the stability of (I) towards acid hydrolysis.

The present work presents three methods for determining (I) in the presence of its acid-induced degradation product using first-derivative spectrophotometry, TLC-densitometry and HPLC. Furthermore, the developed first derivative spectrophotometric method was used to investigate the kinetics of the acid–drug degradation at different temperatures. The three proposed methods were used for determination (I) in commercial tablets without any interference from the excipients normally used in tablet formulations.

2. Experimental

2.1. Instrumentation

A double-beam Shimadzu (Japan) 1601PC UV-visible spectrophotometer connected to a computer fitted with UVPC personal spectroscopy software version 3.7 (Shimadzu) was used. The spectral bandwidth was 2 nm and the wavelength scanning speed was 2800 nm min^{-1}.

The absorbance spectra of test and reference solutions were recorded in 1-cm quartz cells over the range 200–400 nm. The first derivative of the measured spectra were obtained using the accompanying software with $\Delta\lambda = 4$ nm and scaling factor of 10.

The HPLC (Perkin–Elmer, Norwalk, CT, USA) instrument was equipped with a model series 410 LC Pump, Rheodyne 7125 injector with a 20 μl loop and a LC-235 photodiode array detector, separation and quantitation were made on a 150×4.6 mm (i.d.) Phenomenex®. Prodigy 5 μ ODS (5 μm particle size). The detector was set at λ 240 nm. Data acquisition was performed on a model 1022 PE Nelson (Perkin–Elmer).

TLC plates (20×20 cm, aluminum plates precoated with 0.25 mm silica gel F_{254}) were purchased from E.Merck (Darmstadt, Germany). The samples were applied to the TLC plates using 10 μl Hamilton microsyringe. A Shimadzu dual wavelength flying spot densitometer Model CS-9000 was used. The experimental conditions of the measurements were: wavelength = 230 nm, photo mode = reflection, scan mode = zigzag, swing width = 16.

The IR spectrophotometer used was a Shimadzu IR-435.

PMR spectra were recorded on a Varian Gemini 200 PMR spectrometr (200 mHz).

2.2. Materials and reagents

Acebutolol HCl (Rhone Poulenc, Paris, France) was kindly supplied by Alexandria company for pharmaceuticals and chemical industries (Alexandria, Egypt) and used without further purification. The purity of the drug was certified and analyzed to be 99.97%. The water for HPLC was

prepared by double glass distillation and filtration through a 0.45-μm membrane filter. The methanol used was HPLC grade (Honil, UK). Ethanol, chloroform, sodium hydroxide, HCl and acetic acids were analytical grade.

The commercial Sectral tablets used (batch no.6512005), was manufactured by Alexandria Company for pharmaceuticals and chemical industries, under license from Rhone Poulenc, Paris, France, containing acebutolol HCl equivalent to 200 mg acebutolol per tablet.

2.3. Chromatographic conditions

The mobile phase of HPLC was prepared by mixing methanol and water in a ratio of 55:45 v/v. The mobile phase was filtered using a 0.45-μm membrane filter (Millipore, Milford, MA, USA) and degassed by ultrasonic vibrations prior to use. The samples were also filtered using 0.45-μm disposable filters. The flow rate was 1 ml min^{-1}. All determinations were performed at ambient temperature. The injection volume was 20 μl.

The TLC plates were developed in ethanol–glacial acetic acid (4:1, v/v) as a mobile phase. For detection and quantification, 10 μl of test and 10 μl of different concentrations of the standard solutions within the quantitation range were applied as separate compact spots 15 mm apart and 15 mm from the bottom of the TLC plate using 10 μl Hamilton microsyringe. The plate was developed up to the top (over a distance of 15 cm) in the usual ascending way. The chromatographic tank was saturated with mobile phase in the usual mode. After elution the plate was air dried and scanned for acebutolol at 230 nm as described under the instrumental parameters.

2.4. Preparation of the acid-induced degradation product (II)

Accurately weighed 500 mg of (I) were dissolved in 100 ml 0.1 M hydrochloric acid. The solution was refluxed on a heating mantle at 90°C for 20 h. Subsequently, the solution was rendered alkaline with 1 M sodium hydroxide. The degradation product was extracted with chloroform. HCl gas was then passed through the chloro-

formic extract to precipitate the salt. The separated hydrochloride salt was filtered and dried under vacuum. The material was tested for complete degradation using the TLC system described above. A single spot at $R_f = 0.25$ was observed. While no spot was observed at $R_f = 0.52$ corresponding for (I).

The stock solution of (II) was prepared by dissolving 25 mg of (II) prepared as above in 25 ml 0.1 M HCl (for the ^1D method) or methanol (for the TLC and HPLC methods).

2.5. Standard solutions and calibration graphs

2.5.1. For first derivative method
Standard solutions of (I) in the concentration range 10–100 μg ml^{-1} were prepared in 0.1 M HCl. The values of the ^1D amplitudes at 266.6 nm were measured.

2.5.2. For TLC-densitometric method
Standard solutions of (I) in the concentration range 50–1000 μg ml^{-1} were prepared in methanol. Ten microliters of each standard solution were applied to the TLC plates. The plates were chromatographed and the peak areas were measured.

2.5.3. For HPLC method
Standard solutions of (I) in the concentration range 1–10 μg ml^{-1} were prepared in mobile phase. Triplicate 20 μl injections were made for each concentration and chromatographed. The average peak area were calculated.

2.6. Sample preparation

Twenty tablets were weighed and finely powdered. A portion of the powder equivalent to about 100 mg of (I) was weighed accurately, dissolved in and diluted to 100 ml with 0.1 M HCl (for the ^1D method) or methanol (for the TLC-densitometric method) or water (for the HPLC method). The sample solution was then filtered

2.6.1. For first derivative method
The ^1D amplitude was measured for the sample solution containing 50 μg ml^{-1} of (I) at 266.6 nm.

2.6.2. For TLC-densitometric method

Ten microliters of the sample solution containing 500 μg ml^{-1} of (**I**) were applied to the TLC plate and then chromatographed. The concentration of (**I**) in the sample was determined by multilevel calibration developed on the same plate under the same conditions, using second order polynomial regression equation.

2.6.3. For HPLC method

Twenty microliters of the sample solution containing 5 μg ml^{-1} of (**I**) were injected into the HPLC.

2.7. Percent recovery study

This study was performed by adding different amounts of (**I**) and (**II**) to a known concentration of the commercial tablet (standard addition method). The resulting mixtures were assayed and the results obtained were compared with expected results.

2.8. Kinetic study

Accurately weighed 100 mg of (**I**) was transferred into a 250 ml volumetric flask, dissolved and diluted to volume with 0.1 M HCl for each temperature investigation. Separate 25 ml aliquots of this solution were transferred into separate stoppered conical flasks. The flasks were placed in a thermostatic oven at different temperatures (90, 85, 80, 70 and 65°C) for different time intervals (1–6 h). At specified time intervals, the contents of the flasks were transferred into 100 ml calibrated flasks and diluted to volume with 0.1 M HCl. The ^1D amplitude was measured for the solution directly at 266.6 nm and the concentration of the remaining (**I**) was calculated for each temperature and time interval.

3. Results and discussion

3.1. Identification of the degradation product

When (**I**) was heated at 90°C with 0.1 M HCl for 20 h, 3′-acetyl-4′-[2-hydroxy-3-(Isopropylamino)propoxy]aniline dihydrochloride (**II**) and butyric acid (**III**) were produced (Scheme 1). Using the above mentioned TLC system, the R_f values of the compounds were found to be 0.52 and 0.25 for (**I**) and (**II**), respectively.

The structure assigned for product (**II**) was confirmed through its diazotization, then coupling with β-naphthol solution, where an orange color was produced, indicating the presence of a free primary amino group.

A conclusive proof of structure (**II**) was based on comparing of the PMR spectral data of a purified specimen (**II**), separated from the degradation reaction, with that of the intact compound (**I**). The PMR spectrum of (**I**) in DMSO was characterized by the appearance of a D$_2$O-exchangeable amide proton at δ 5.94 ppm and the protons of the butyroyl residue at δ 0.87–0.94 ppm (triplet, 3H, C$\underline{H}_3$–CH$_2$–); δ 1.55–1.66 ppm (sextet, 2H, CH$_3$–C$\underline{H}_2$–CH$_2$–); and δ 2.24–2.31 ppm.(triplet,2H, CH$_3$–CH$_2$–C$\underline{H}_2$–CONH). By contrast, the PMR spectrum of (**II**) in the same solvent lacked the characteristic amide and the butyroyl proton signals of (**I**).

The IR spectrum (KBr) of (**II**) showed the appearance of a band at 3550 cm^{-1} corresponding to the primary amine group and disappear-
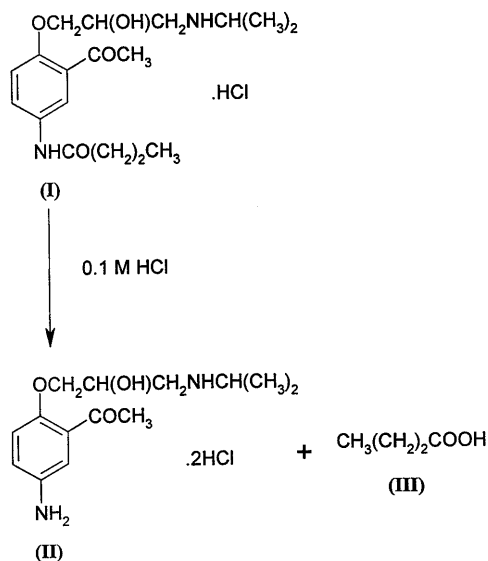
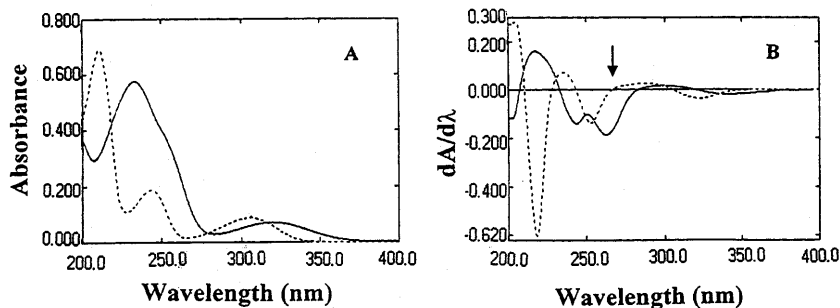Scheme 1. Suggested degradation pathway for acebutolol HCl in 0.1 M HCl.

Fig. 1. UV absorption spectra (A) and first derivative spectra (B) of 10 μg ml^{-1} of both acebutolol HCl (—) and 3′-acetyl-4′-[2-hydroxy-3-(Isopropylamino) propoxy] aniline dihydrochloride (- - -) in 0.1 M HCl.

ance of the band at 1660 cm^{-1} assigned to the stretching absorption of the C = O of the amide. Other characteristic stretching absorption bands such as the OH, the NH of the secondary amine and the C = O of the ketone retained their positions at 3420, 3350 and 1680 cm^{-1}, respectively.

3.2. Assay parameters

3.2.1. First derivative UV spectrophotometric method

The UV absorption spectra of (I) and (II) in 0.1 M HCl are overlapped (Fig. 1A), while their first derivative spectra (Fig. 1B) showed significant differences in some areas that permits the determination of (I) in the presence of (II). (I) was determined by measurement of its first derivative amplitude at the zero-crossing point of (II) (at 266.6 nm). The plot of the absolute value of first derivative at 266.6 nm against concentration of (I) in the range of 10–100 μg ml^{-1} showed a linear relationship. The regression equation was found to be: $^1D = -14.2 \times 10^{-3} + 19.8 \times 10^{-3} C$, ($r = 0.9998$), where ^1D is amplitude at 266.6 nm and C is the concentration of (I) in μg ml^{-1}.

In general, the characteristic profiles of the derivative spectra may constitute a specific fingerprint useful for the drug identification, in particular, the ratios between the amplitudes at selected wavelength can be regarded as suitable parameters which are useful to confirm the drug identity, purity and stability [21]. The ratio of the values of $^1D_{243.3}/^1D_{262.3}$ of (I) was found to be 0.745 with relative standard deviation of 0.39% ($n = 6$). This

figure was altered in the presence of (II) in the sample.

3.2.2. TLC-densitometric method

Instrumental planar chromatography with precise sample application and computer-controlled evaluation and quantification of the developed chromatograms has been considered as reliable tool for purity control and quantitative drug testing [22]. Experimental conditions, such as mobile phase, scan mode and wavelength of detection, were optimized to provide accurate, precise and reproducible results for determination of (I) in the presence of (II). The chosen scan mode was the zigzag mode and the wavelength of scanning was chosen to be 230 nm. The greatest differences between the R_f values of the two compounds (0.52 and 0.25 for (I) and (II), respectively), were obtained by the system containing ethanol–glacial acetic acid in ratio of 4:1 v/v, respectively.

The relationship between the concentration of (I) and peak area of the spot was investigated. The linear relationship was tested and the correlation coefficient was found to be 0.996 for 11 points. However, examination of the residuals showed some curvature, indicating bad correlation. The second-order polynomial fit was found to be more suitable, its residuals plot showed a much better even scatter than that of linear model, indicating good correlation. The calibration graph was constructed in the range 0.5–10 μg/spot. The second-order polynomial regression equation was found to be: $A = -1.33 \times 10^2 C^2 + 5.20 \times 10^3 C + 1.79 \times 10^3$, ($r = 0.9998$), where A is

the peak area of the spot and C is the concentration of (**I**) in µg/spot.

3.2.3. HPLC method

The developed HPLC method has been applied for the determination of (**I**) in the presence of (**II**). To optimize the HPLC assay parameters, the effect of methanol composition of the mobile phase on the capacity factor (K') was studied. A satisfactory separation was obtained with a mobile phase consisting of methanol–water mixture (55:45, v/v). Increasing methanol concentration to more than 75% led to inadequate separation of (**I**) and (**II**). At lower methanol concentration, separation occurred but with excessive tailing and increased retention time for (**I**) peak.

The specificity of the HPLC method is illustrated in Fig. 2 where complete separation of (**I**) and (**II**) was noticed. The average retention time ± standard deviation for (**I**) and (**II**) were found to be 3.7 ± 0.020 and 2.1 ± 0.024 min, respectively, for 10 replicates. The peaks obtained were sharp and have clear baseline separation.

To determine the linearity of the HPLC detector response, calibration standard solutions of (**I**) were prepared as described in the text. Linear correlation was obtained between peak area versus concentration of (**I**) in the range of 1–10 µg ml^{-1}. Each measurement represented the average of three replicates. The regression equation was found to be: $A = -4.49 \times 10^3 + 3.38 \times 10^5 C$, ($r = 0.9998$), where A is the peak area and C is the concentration of (**I**) in µg ml^{-1}

3.3. Accuracy and selectivity of the proposed method

The three proposed methods are highly selective towards acebutolol HCl (**I**) and 3′-acetyl-4′-[2-hydroxy-3-(Isopropylamino) propoxy] aniline dihydrochloride (**II**). Regarding butyric acid (**III**), it is assumed that its UV absorption characteristics are relatively low to be detected by the assay conditions used in this work.

The accuracy of the three proposed methods was checked by analyzing different laboratory-prepared mixtures of (**I**) and (**II**) at various concentrations ranged from 40 to 90 µg ml^{-1} for (**I**) and 10 to 50 µg ml^{-1} for (**II**) (for ^1D method); from 3 to 9 µg/spot for (**I**) and 1 to 7 µg/spot for (**II**) (for TLC method); and from 2 to 8 µg ml^{-1} for (**I**) and 0.5 to 5 µg ml^{-1} for (**II**) (for HPLC method). The mean percentage recovery of (**I**) ± relative standard deviation (RSD) were found to be 100.4 ± 0.73, 100.3 ± 0.90 and 99.8 ± 0.42 for ^1D, TLC and HPLC methods, respectively. The above mentioned three methods were able to determine (**I**) in presence of (**II**) in ratios up to 1:1,1: 2.33 and 1: 0.63, respectively. This indicates the high repeatability and accuracy of the three methods.

The detection limits of (**I**) in the three proposed methods were found to be 0.55, 0.32 and 0.03 µg ml^{-1} for ^1D, TLC and HPLC methods, respectively. The relative sensitivity, based on detection limit, was calculated. The HPLC method was found to be more sensitive than the ^1D and TLC methods; and the TLC method was found to be more sensitive than the ^1D method.
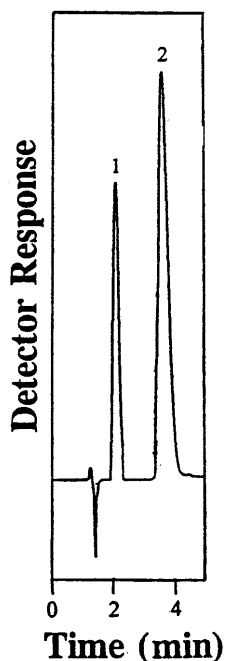

Fig. 2. A typical chromatogram of 20 µl injection of synthetic mixture of 10 µg ml^{-1} of both acebutolol HCl (2) and 3′-acetyl-4′-[2-hydroxy-3-(Isopropylamino) propoxy] aniline dihydrochloride (1).

3.4. Tablet analysis

The three proposed methods were applied to the determination of (**I**) in commercial tablets. The mean percentage $\pm$ standard deviation ($n = 9$) were found to be 100.1 ± 0.52, 100.1 ± 0.68 and 100.2 ± 0.53, determined by ^1D, TLC and HPLC methods respectively. Moreover, to check the validity of the proposed methods, the standard addition method was applied by adding different amounts of (**I**) with concentration range $20–40$ $\mu g\,ml^{-1}$ (for ^1D), $1–6$ μg spot^{-1} (for TLC) and $2–7$ $\mu g\,ml^{-1}$ (for HPLC); and (**II**) with concentration range $10–50$ $\mu g\,ml^{-1}$ (for ^1D), $1–5$ μg spot^{-1} (for TLC) and $2–5$ $\mu g\,ml^{-1}$ (for HPLC) to the previously analyzed tablets. The mean recovery of the added (**I**) $\pm$ relative standard deviation were found to be 100.2 ± 0.57, 100.0 ± 0.72 and 100.2 ± 0.36 for ^1D, TLC and HPLC methods, respectively. The results of analysis of the commercial tablets and the recovery study (standard addition method) suggested that there is no interference from any excipients which are normally present in tablets.

The results of determination of (**I**) in tablets obtained from the ^1D and TLC methods were compared with the HPLC method. Statistical comparison of the results was performed with regard to accuracy and precision using Student's t-test and the F-ratio at 95% confidence level. The calculated Student's t-test and F-ratio values were found to be 0.40 and 1.04 ($n = 9$) (for ^1D and HPLC comparison); 0.35 and 1.65 ($n = 9$) (for TLC and HPLC comparison), while the theoretical values are 2.12 and 3.44, respectively. It is clear that there is no significant difference between the three methods with regard to accuracy and precision.

3.5. Kinetic investigation

To assess the specificity and selectivity of the ^1D method for the assay of (**I**) without interference from (**II**), acid-degradation of acebutolol HCl was carried out under the previously described experimental conditions. A regular decrease in the concentration of (**I**) with increasing time intervals was observed. At the selected temperatures

Table 1
Degradation rate constant (K_{obs}) and half life ($t_{1/2}$) for acebutolol HCl in 0.1 M HCl.

Temperature (°C)	K_{obs} (h^{-1})	$t_{1/2}$ (h)
90	0.16	4.36
85	0.11	6.50
80	0.07	9.93
70	0.03	22.80
65	0.02	34.59

(90,85,80,70 and 65°C), the acid-degradation process followed pseudo first-order kinetics. From the slopes of the straight lines it was possible to calculate the apparent first-order degradation rate constant and the half life at each temperature, determined by ^1D method (Table 1). Plotting log K_{obs} values versus $1/T$, the Arrhenius plot (Fig. 3) was obtained, which was found to be linear in the temperature range 65–90°C. The activation energy was calculated to be 19.8 Kcal mol^{-1}, which is comparable to activation energy of acid hydrolysis of amide bond of captopril (21.4 Kcal mol^{-1}) [23]. This data indicates that (**I**) is relatively stable to acid hydrolysis.

4. Conclusion

The proposed ^1D, TLC and HPLC methods provide simple, accurate and reproducible quantitative analysis for the determination of acebutolol HCl in pharmaceutical tables and in the presence of its acid-induced degradation product. The HPLC method was found to be more sensitive
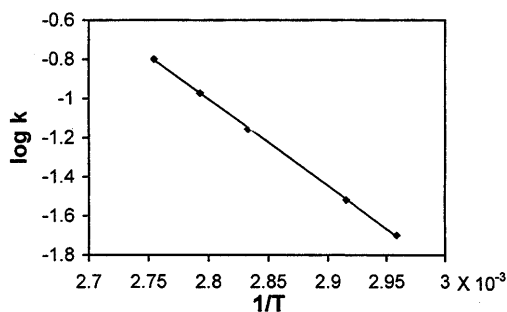

Fig. 3. Arrhenius plot for the degradation of acebutolol HCl in 0.1 M hydrochloric acid using ^1D method.

than the ^1D and TLC methods. While the ^1D method has the advantages of lower cost, rapid and environment protecting. The TLC method is simple and uses a minimal volume of solvents, compared to the HPLC method. The three proposed methods are suitable for quality control laboratories, where economy and time are essential.

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