Energy-dependent electrospray ionization mass spectrometry (for Vol 6 of EMS)

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1. Introduction

Collision-induced dissociation (CID; sometimes CAD, see Volume 1, Chapter 6, *Collisional Activation for Structural Analysis*) of ions generated by the electrospray process may be carried out in-source or in a collision cell. When performed in-source, fragment ions are generated from all ions present, and the resulting spectrum is correspondingly complicated. To obtain fragment ions from a single precursor-ion, the appropriate ion is selected by an initial stage of mass analysis, fragmented in a collision cell and the product-ion mass spectrum collected. In both cases, the degree to which the ions are fragmented by CID depends on the extent to which the ions are accelerated, the *m/z* value of the ion, and the nature of the colliding target itself (see Volume 1, Chapter 6, *Collisional Activation and Dissociation: Methodology*). As a result, the appearance of the spectrum is depends highly on the experimental conditions. The conditions are usually "tweaked" to maximize the information content of the spectrum; a typical approach is to set the voltages such that the precursor-ion signal intensity is reduced to a fraction (say 25%) of the intensity of the base peak. The majority of ESI product-ion spectra are depicted in this way. However, there are examples in which spectra collected for the same compound but under different conditions show entirely different collections of product ions, and multiple spectra must be presented to insure that all fragment ions are represented [1]. A common way to present the complete ensemble of fragment ions is to stack spectra collected under increasingly energetic conditions, as shown in Figure 1 for the fragmentation of $[Fe(CO)_3(\eta^5\text{-}C_6H_7)]BF_4$.

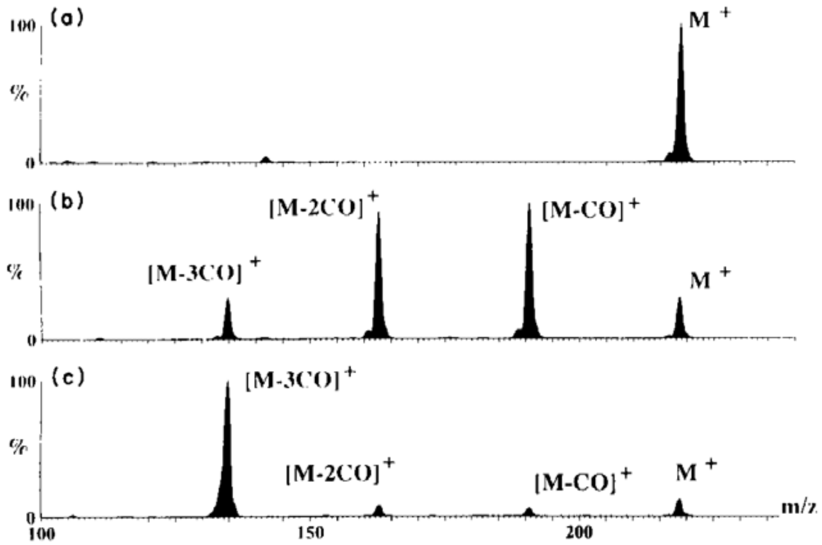


Figure 1. Positive-ion ESI mass spectra of $[Fe(CO)_3(\eta^5\text{-}C_6H_7)]BF_4$ at skimmer voltages of (a) 18 V; (b) 50 V; (c) 100 V. Reproduced from reference 1 with permission.

A stacked display has some disadvantages; the conditions are usually chosen arbitrarily, the number of spectra required will vary from sample to sample, interpretation of several spectra simultaneously is not especially intuitive, and frequently spectra are discarded on the basis that they are unrepresentative. An alternative is to collect spectra across the entire range of available voltages and to present this complete fragmentation information in a single spectrum. Simply summing all these spectra generates a spectrum in which all product ions and the precursor ion appear. However, this approach discards an entire dimension of data; namely, the voltage profile for the appearance and disappearance of an ion. This information can be extracted experimentally by collecting spectra at different energies and plotting ion signal intensity in the form of a breakdown graph, showing all the ions of interest.

2. Breakdown Graphs

Figure 2 shows the breakdown plot for the cation of an ionic liquid, in which ten fragment ions are plotted on a logarithmic scale (greatly enhancing the prominence of the less abundant ions) [2].

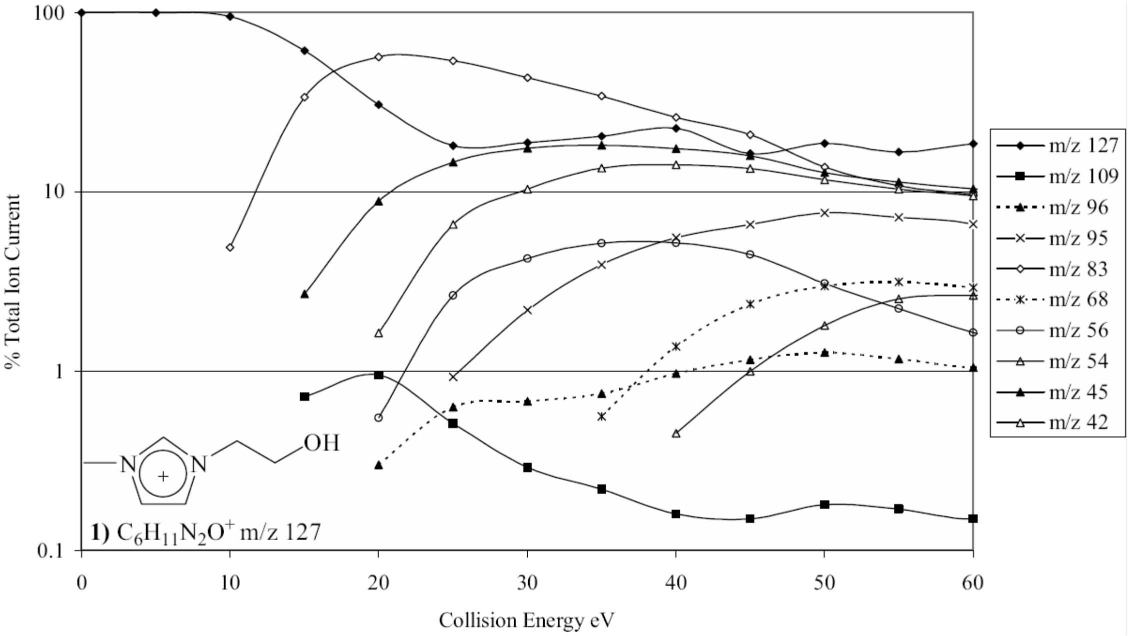


Figure 2. Breakdown graph for an ionic liquid. Solid lines represent even electron ions, dotted lines represent odd electron ions. Reproduced with permission from reference 2.

Breakdown plots allow the analysis in detail of fragmentation of compounds as diverse as complex inorganic ions [3], crown ethers, nucleic acid bases [4], and amino acids [5]. Experiments involving the construction of breakdown plots are often described as energy-resolved mass spectrometry [6] (sometimes energy-variable mass spectrometry) [7]. There is an entire subfield of mass spectrometry devoted to the establishment of thermodynamic data from CID studies under single-collision conditions (see Volume 1, Chapter 6, *Collisional Activation and Dissociation: Single Collisions*).

3. Energy-dependent electrospray ionization (EDESI)

EDESI [8] is an approach that retains *all* of the information from a detailed CID study and presents it in an easily-digestible format. Spectra are collected over the full range of available CID energies and are used to create a three-dimensional depiction of the data. One dimension is the mass-to-charge ratio (*m/z*), another is the ion intensity (%), and the third is the CID energy (typically “cone” or “collision” voltage). This information may be plotted in a variety of ways, for example as a 3D surface (Figure 3), or more usually, as a 2D contour plot (Figure 4).

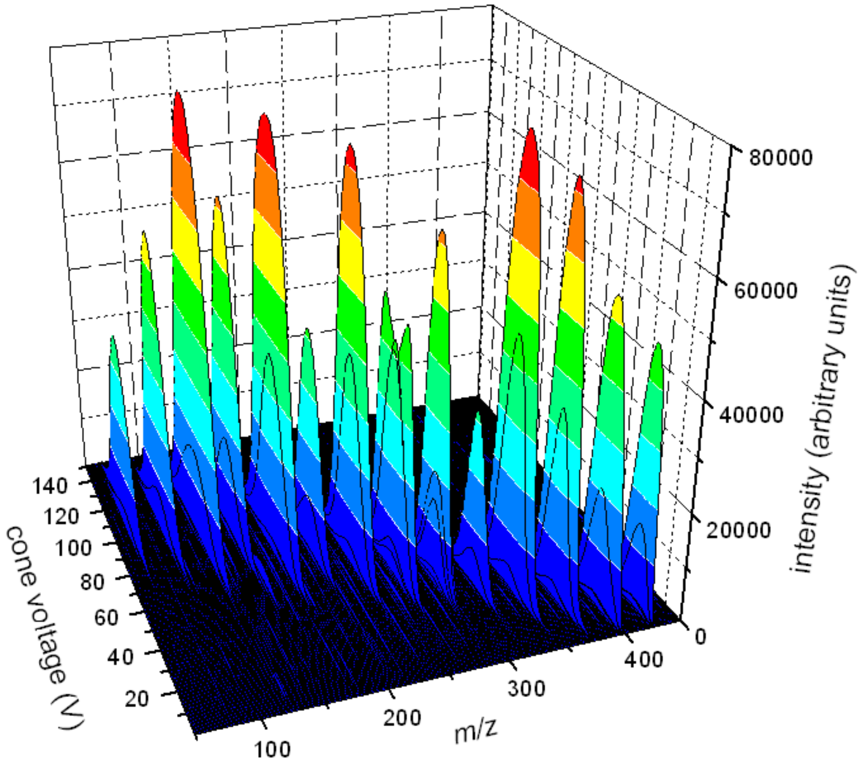


Figure 3. A 3D surface representation of the EDESI-MS data for the ion $[Fe_3H(CO)_{11}]^-$, obtained in negative-ion mode with methanol as the mobile phase. Reproduced with permission from reference 19.

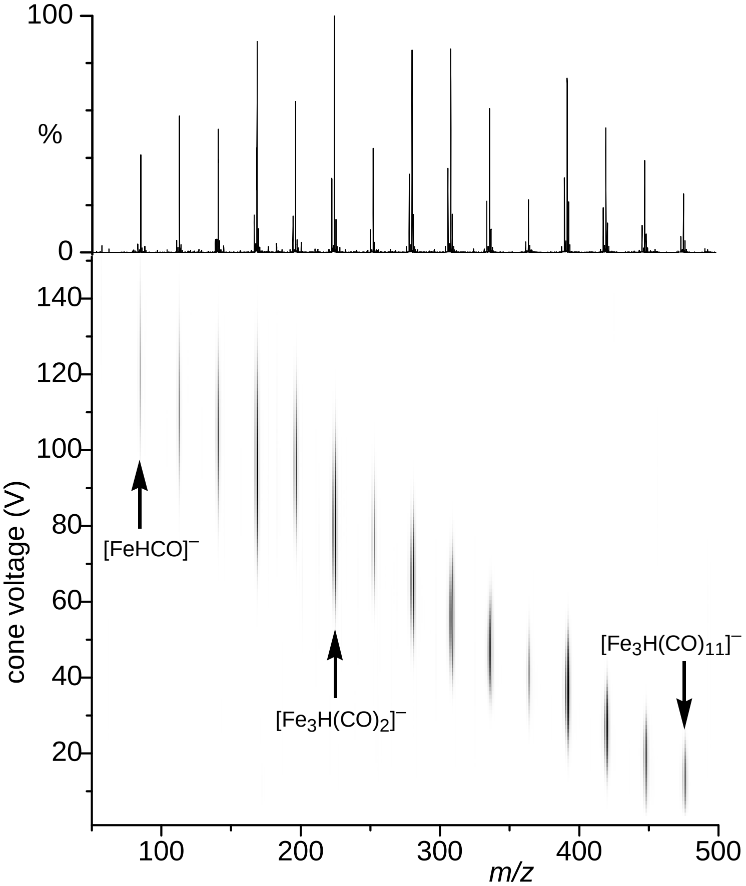


Figure 4. 2D map EDESI-MS of $[Fe_3H(CO)_{11}]^-$, run in negative ion mode with methanol as the mobile phase. The top spectrum is a summation of the 151 spectra used to generate the map. Reproduced with permission from reference 19.

Breakdown plots may be incorporated into the presentation with ease [9], and where the CID occurs in the collision cell rather than at the cone, the term EDESI-MS/MS is appropriate [10]. Both of these features are evident in Figure 5, a full analysis of the CID behavior of protonated cysteine.

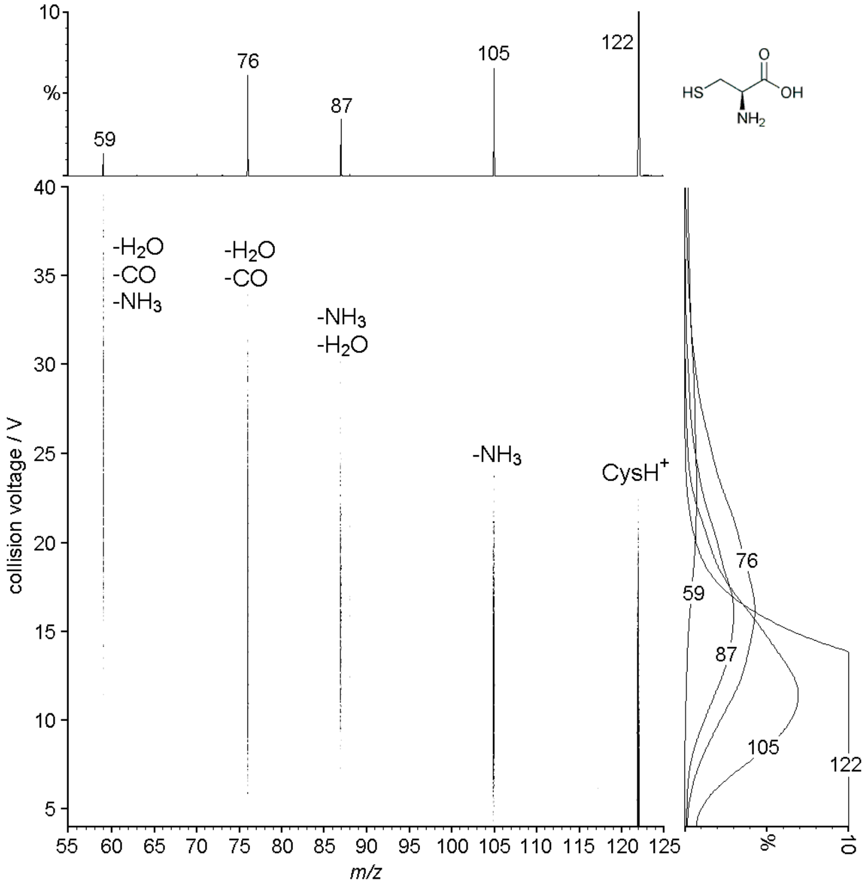


Figure 5. The EDESI-MS/MS generated from 360 positive product-ion mass spectra of [cysteine + H]$^+$ at collision voltage settings of 4 - 40 V (increments of 0.1 V). The top trace is a 1D spectrum generated by combining all 360 spectra. The side trace is a breakdown graph of all the major ions in the spectrum. Reproduced by permission from reference 9.

The primary intention behind conducting an EDESI-MS experiment is to gather detailed information on ion structure using CID and render it, in its entirety, in a format that is intuitive and easy to interpret. For simple compounds, like cysteine (Figure 5), which decompose into only a handful of fragment ions that, under appropriate conditions, may all appear simultaneously in the spectrum, an EDESI-MS approach to interpretation can fairly be described as overkill. However, for more complicated compounds – particularly inorganic and organometallic complexes (see this volume 1, chapter 21, *Organometallics*) – spectra are frequently dramatically different when conditions are changed, and the EDESI-MS approach is a great aid to understanding the processes at work. This point is best illustrated with an example.

4. Some Applications

Metal carbonyl clusters, $M_n(CO)_m$, fragment primarily through loss of CO ligands as carbon monoxide [11]. Clusters that carry a charge (usually anionic) provide excellent ESI spectra [12]. For the most part, CO ligands are stripped off in very regular intervals as the CID energy is increased, and the EDESI mass spectra of these compounds are fairly simple. For clusters with an open core geometry (i.e., the number of metal-metal bonds is not at the maximum value), the EDESI spectrum is more complicated. The cluster $[Pt_3Ru_{10}C_2(CO)_{32}]^{2-}$ undergoes fragmentation by two competing paths (Figure 6) [13]. Both begin with the loss of a single carbonyl ligand, and one of the paths involves a continuation of this process. The intensity of the CO-loss peaks at low cone voltages is highly variable, and this feature is attributable to the ability of the cluster to rearrange and form more M-M bonds to compensate for the loss of the CO ligands. Once the number of M-M bonds has reached a maximum, the intensities of all subsequent CO loss fragments are approximately equal. Multiply-charged anions have a limited existence in the gas phase [14], and electron autodetachment occurs late in the fragmentation process to form monoanionic fragments (not shown in Figure 6) [15]. The other pathway involves the cluster splitting into two *monoanionic* fragments, $[PtRu_5C(CO)_{15}]^-$ and $[Pt_2Ru_5C(CO)_{16}]^-$, which appear in the spectrum at *m/z* values slightly lower and slightly higher, respectively, than the original cluster (*z* in the *m/z* term has been halved). These monoanionic fragments undergo CO loss down to $[PtRu_5C]^-$ and $[Pt_2Ru_5C]^-$ cores, respectively. The fragmentation processes are summarized in Scheme 1.

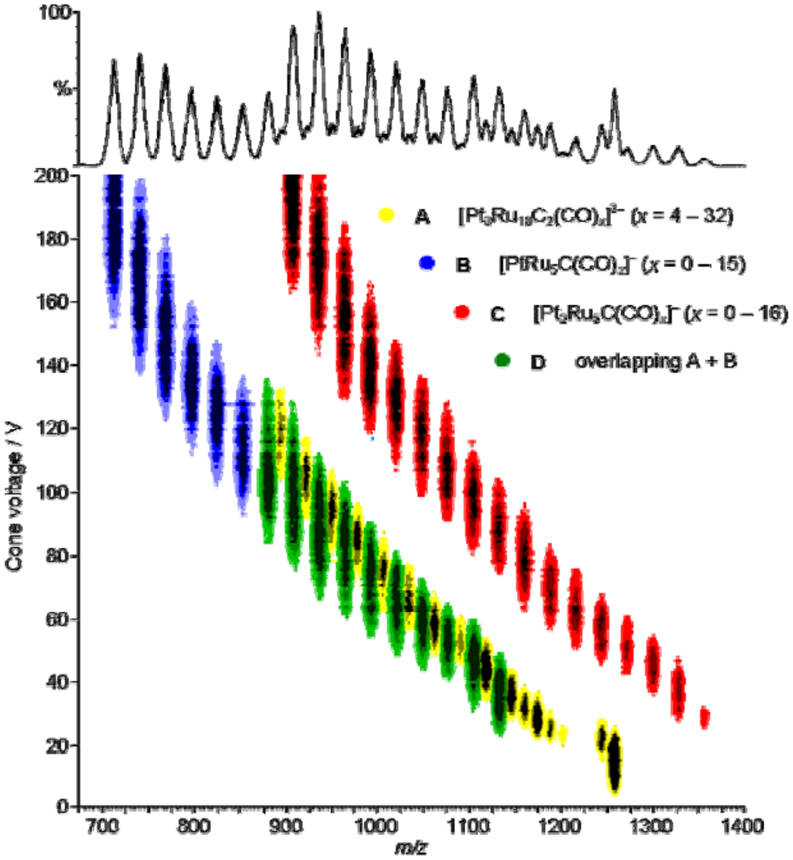
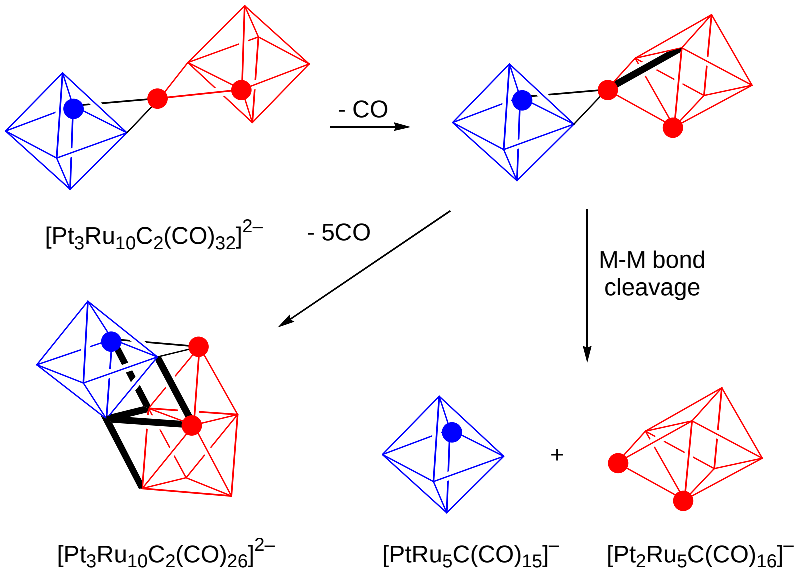


Figure 6. Part of the negative-ion EDESI-MS of the transition metal carbonyl cluster dianion $[Pt_3Ru_{10}C_2(CO)_{32}]^{2-}$. Adapted from reference 13 with permission.



Scheme 1. The two principal fragmentation pathways for $[Pt_3Ru_{10}C_2(CO)_{32}]^{2-}$. Adapted from reference 13 with permission.

Mononuclear metal carbonyls can be derivatised with methoxide ion to form species of the type $[M(CO)_n + OMe]^-$, and their CID studies using EDESI-MS are available [16]. Fragmentations involving both CO and HCHO losses occur. EDESI-MS can also probe oxygen-atom transfer pathways in molybdenum complexes [17].

EDESI-MS is a powerful tool for the interpretation of CID spectra of complex mixtures. Figure 7 shows the results of an experiment conducted on the mixture of products formed from the reaction of $[Co(CO)_4]^-$ with $Ru_6C(CO)_{17}$ [18]. At low cone voltages, four signals are observed, corresponding to $[Ru_5CoC(CO)_{16}]^-$, $[Ru_3Co(CO)_{13}]^-$, $[RuCo_3(CO)_{12}]^-$, and $[HRu_4Co_2C(CO)_{15}]^-$. CID at the skimmer cone simultaneously fragments all four species, generating a wealth of daughter ions, whose parentage is immediately apparent from the EDESI mass spectrum. This type of study is particularly advantageous when using an instrument without MS/MS capability. Those who do have access to MS/MS can perform separate EDESI experiments on each parent ion, to eliminate any ambiguity with respect to which product ion belongs to which precursor.

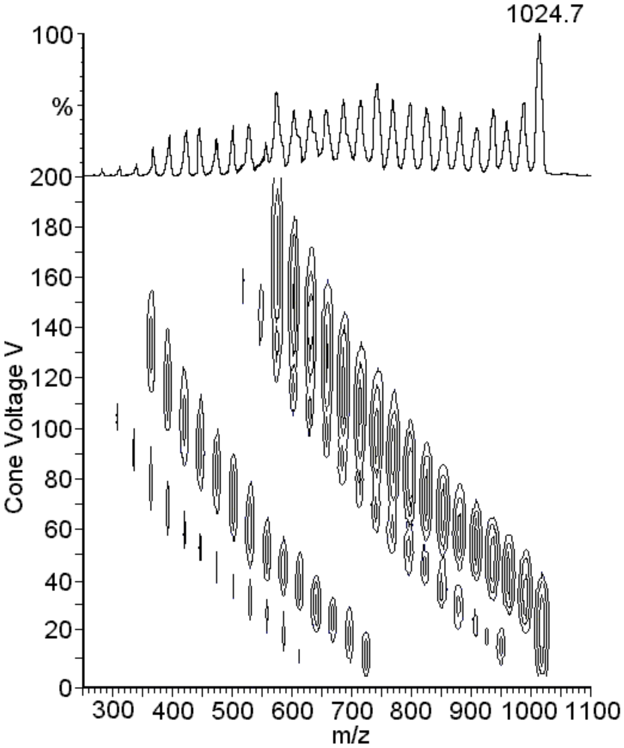


Figure 7. Negative-ion EDESI-MS of the mixture of products from the reaction of $[Co(CO)_4]^-$ with $Ru_6C(CO)_{17}$. Adapted from reference 18 with permission.

One barrier to the wider adoption of the EDESI-MS approach is the unavailability of commercial software for the generation of spectra. However, this lack has been at least partially addressed by the development of the program "EDit" [19], which takes raw spectra and converts them into a single matrix suitable for processing by any of the many commercial scientific graphing packages.

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