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PCI-like behavior of estradiol in MALDI

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To the Editor

Sir,

Ordinarily only labile analytes undergo significant prompt fragmentation in matrix-assisted laser desorption ionization-mass spectrometry (MALDI-MS),¹ such as oligonucleotides² or vitamin B₁₂.³ Basically this is because MALDI, which commonly uses moderate laser fluences, is a relatively gentle ionization/desorption technique. It was therefore of interest for us to observe a relatively abundant fragment ion at m/z 255 from β -estradiol (E_2 , M_r = 272, structure in Figure 1), under routine MALDI conditions, with α -cyano-4-hydroxycinnamic acid (CCA) as matrix, as seen in Figure 2A. We wondered whether the ion at m/z 255 came from loss of water from protonated E_2 (m/z 273), or from loss of a hydroxyl radical from the radical cation (m/z 272), and how this took place. (Note that the peak at m/z 273 in Figure 2A is mostly (81%) the ¹³C form of the radical cation at m/z 272, and partly (19%) protonated E_2 .) Our attention immediately turned to the former pathway since electron ionization of E_2 at 70 eV gives negligible loss of OH.⁴ Further, subjecting m/z 272 to post-source decay furnished only a negligible product ion at m/z 255, as seen in Figure 2B.

In principle, protonated E_2 might lose water in four ways: an elimination reaction (loss of OH along with an adjacent H) at C3 or C17, or a non-elimination reaction at either of these sites. In a post-source decay experiment we observed that the ion at m/z 255 retains the aryl-OH: the major product ion from m/z 255 is m/z 159, and the aryl-OH moiety is part of this ion (Figure 3A). This turned our attention to the loss of water at C17. An elimination mechanism for the loss of water at this carbon atom (in conjunction with protonation at the aryl-OH to yield an ion of m/z 255) was ruled out by the observation that m/z 255 is completely replaced by m/z 258 when 17 β -estradiol-16,16,17- d_3 (structure shown in Figure 1, prepared by Prime Organics, Woburn, MA, USA) is tested instead of E_2 , as seen in Figure 2C. (We also studied 17 β -estradiol-16,16,17- d_3 by post-source decay, as shown in Figure 3C, to strengthen the assignments that we made for the ions shown in Figure A3A.) A mechanism peculiar to CCA was ruled out by the observation that the product ion at m/z 255 also forms readily when using a matrix of 2,5-dihydroxybenzoic acid, as seen in Figure 2D. (Note that in the latter spectrum the intense peak at m/z 273.044 is a matrix ion, while m/z 273.171 is the combination of the ¹³C of the radical cation and the protonated molecule.) The analogous estrogen, 5 α -androstane-17 β -ol, readily loses OH in positive chemical ionization (PCI) with methane.⁵ Alcohols in general (at least those with five or more carbon atoms) readily lose OH in PCI.⁶ We observed that 5 β -androstane-17 β -ol (M_r = 276,

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Steraloids Inc., Newport, RI, USA) readily losses OH in MALDI, actually with no analyte peak at all at m/z 276 or 277. The behavior of E_2 in MALDI can therefore be described as “PCI-like.” There is much evidence that energetic proton transfer reactions are important in MALDI, arising either from electronically excited states of matrix molecules, or from radical cations formed by the intense laser irradiation of the matrix.⁷ Matrix cation radicals have proton affinities of about 8.5 eV^{8,9}, whereas the proton affinity of CH_4 is about 5.7 eV. Thus, if the proton donor to E_2 in MALDI is a matrix cation radical, other energies (e.g. vibrational) may be playing a role. This would not necessarily be true if the donor is a matrix metastable, or a relatively free proton.

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Figure 1.
Structures of the steroids tested by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS).

Figure 2. MALDI-TOF mass spectra of E₂ or isotopic E₂. **A**, E₂, matrix is CCA; **B**, PSD of E₂ precursor ion m/z 272.5, CCA; **C**, E₂-16,16,17-d₃, CCA; **D**, E₂, DHB

Figure 3.

Post source decay spectra by MALDI-TOF-MS in CCA. **A**, E₂, with m/z 255 as the selected precursor ion; **B**, E₂-16,16,17-d₃, with m/z 258 as the selected precursor ion.