To aid in the identification and quantification of biologically and agriculturally significant natural products, tandem mass spectrometry can provide accurate structural information with high selectivity and sensitivity. In this study, diagnostic fragmentation patterns of isoflavonoids were examined by liquid chromatography-ion trap-time of flight-mass spectrometry (LC-IT-TOF-MS). The fragmentation scheme for $[M+H-2CO]^+$ ions derived from isoflavones and $[M+H-B\text{-ring}-CO]^+$ ions derived from 5-hydroxyisoflavones, were investigated using different isotopically labeled isoflavones, specifically [1′,2′,3′,4′,5′,6′,2,3,4-$^{13}C_9$] and [2′,3′,5′,6′,2-D_5] isoflavones. Specific isotopically labeled isoflavones were prepared through the biosynthetic incorporation of pharmacologically applied ^{13}C- and D-labelled L-phenylalanine precursors in soybean plants following the application of insect elicitors. Using this approach, we empirically demonstrate that the $[M+H-2CO]^+$ ion is generated by an intramolecular proton rearrangement during fragmentation. Furthermore, $[M+H-B\text{-ring}-CO]^+$ ion is demonstrated to contain a C_2H moiety derived from C-ring of 5-hydroxyisoflavones. A mechanistic understanding of characteristic isoflavone fragmentation patterns contributes to the efficacy and confidence in identifying related isoflavones by LC-MS^n.

The fragmentation pattern of isotopically labeled isoflavones, which were prepared by the treatment with the combination of isotopically labeled phenylalanine and insect-derived elicitors, was investigated by LC-IT-TOF-MS.