

Nitrogenous flavonoid derivatives production by reacting with amino acids

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A class of nitrogenous derivatives of flavonoids was detected using *in vitro* cell culture medium and *in vivo* mice produced without enzymatic catalysis have been consistently overlooked in literature. In this study, 39 flavonoids were incubated in Dulbecco's modified eagle's medium (DMEM) at 37°C for 2 h to explore the reaction mechanism behind nitrogenous derivatives from flavonoids. Baicalein, scutellarein, DMY, GC, EGC, and EGCG were found to produce corresponding nitrogenous derivatives in both DMEM and mixed amino acid solution. The nitrogen source of these 6 flavonoid nitrogenous derivatives was revealed to be amino acid. The reaction site with amines in these flavonoid nitrogenous derivatives was identified as OH of the pyrogallol moiety in flavonoids *via* LC-MS/MS and NMR. This pyrogallol group was a key motif being first oxidized into quinone, further, to react with Strecker degradation of amino acids to yield N-flavonoids and corresponding aldehydes. Reaction optimization revealed that a slightly alkaline environment accelerates flavonoid nitrogenous derivatives formation by promoting the formation of flavonoid quinone. These results provide the first mechanistic evidence for the *in vitro* generation of flavonoid nitrogenous derivatives yet to be tested using *in vivo* assay.

Keyword: Nitrogenous flavonoids; baicalein; dihydromyricetin; catechin; pyrogallol; amino acids

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