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Transformation of Arctiin to Estrogenic Substances by Human Intestinal Bacteria

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[Objective] Phytoestrogens, including lignans and isoflavonoids, irrespective of beneficial or deleterious effects, are conceived to play an important role in hormone-dependent disease, such as breast cancer and coronary heart disease. Arctiin (a main lignan in *Arctium lappa*) was reported to have protective effect on 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) induced mammary carcinogensis in female rats by oral administration. Arctiin can be transformed to two metabolites by rat intestinal flora. The present study was designed for better understanding of the metabolism of arctiin by human intestinal bacteria and its relationship to estrogenic activity.

[Method] Arctiin (1g) was anaerobically incubated with a 0.5% human fecal suspension (in 2000 ml GAM broth) in an anaerobic incubator for 9 days. Every 100 ml reaction mixture was taken out at intervals followed by extracting with *n*-BuOH. Metabolites were isolated from the *n*-BuOH extract by repeated column chromatography. The structures were determined by EI-MS, 1D and 2D-NMR. Arctiin and its metabolites were evaluated for estrogenic activity by effects on the growth of human breast cancer MCF-7 cells in culture.

[Results and Conclusion] Six metabolites of arctiin by human intestinal bacteria were isolated and identified as arctigenin (AM1), 2-(3',4'-dihydroxybenzyl)-3-(3",4"-di methoxy benzyl) butyrolactone (AM2), 2-(3'-hydroxybenzyl)-3-(3",4"-dimethoxy benzyl) butyrolactone (AM3), 2-(3'-hydroxybenzyl)-3-(3"-hydroxy-4"-methoxybenzyl) butyrolactone (AM3-M1), 2-(3'-hydroxybenzyl)-3-(3",4"-dihydroxybenzyl) butyrolactone (AM4) and enterolactone (AM5). We isolated AM3, AM3-M1, AM4 and enterolactone for the first time as metabolites of arctiin, and AM3-M1 was a new compound. A possible metabolic pathway was put forward according to the time course of the transformation. Incubation of arctiin with a rat fecal suspension led to two metabolites, arctigenin and AM2, which may be reflected the difference in bacteria flora between rats and humans. On the growth of MCF-7 human breast cancer cells in culture, AM4 showed the most potent proliferative effect among arctiin and six metabolites. Enterolactone showed proliferative effect only at high concentrations (10^{-5} M), while AM4 showed high activity even at low concentrations (10^{-6},10^{-7},10^{-8} M). These results indicate that the biotransformation by intestinal bacteria is essential for the manifestation of estrogenic activity of arctiin.