Hydroxybenzophenones absorb and dissipate ultraviolet (UV) A light, and are used as UV stabilizers and sunscreens. Benzophenone-3 (BP-3; 2-hydroxy-4-methoxybenzophenone) is a widely used sunscreen component, which shows weak estrogenic activity. In this study, we examined the in vitro metabolism of BP-3 by rat liver microsomes and the effect of the metabolism on the estrogenic activity. BP-3 was incubated with liver microsomes of untreated, phenobarbital (PB)-treated and 3-methylcholanthrene (MC)-treated rats in the presence of NADPH, and metabolites formed were analyzed by means of HPLC. Estrogenic activity was examined using the MCF-7 luciferase reporter assay. When BP-3 was incubated with liver microsomes of MC-treated rats, one major metabolite (M-1) and three minor metabolites (M-2, M-3 and M-4) were detected. M-1 was identified as 2,5-dihydroxy-4-methoxybenzophenone by comparison with an authentic sample, and the other metabolites were identified as 2,4,4′-trihydroxybenzophenone (M-2), 2,4-dihydroxybenzophenone (M-3) and 2,2′-dihydroxy-4-methoxybenzophenone (M-4). Metabolism of BP-3 to M-1 and M-2 was catalyzed by CYP1A1, 2C6 and 3A1, and by CYP2C6, respectively. The estrogenic activity of extracts after incubation with liver microsomes of PB- and MC-treated rats was much higher than that of BP-3. 2,3,4-Trihydroxybenzophenone exhibited the highest estrogenic activity among the metabolites, followed by 2,4-dihydroxybenzophenone, BP-3, 2,5-dihydroxy-4-methoxybenzophenone and 2,2′-dihydroxy-4-methoxybenzophenone. These results suggest that the estrogenic activity of BP-3 is activated by conversion to the desmethylated metabolites, 2,4,4′-trihydroxy- and 2,4-dihydroxybenzophenone.