A new fumonisin, fumonisin B-6 (1), has been isolated by cation-exchange and reverse-phase chromatography, together with fumonisin B-2 (2), from stationary cultures of the fungus Aspergillus niger NRRL 326. Analysis of mass spectrometric and NMR data determined that FB6 is a positional isomer of FBI and iso-FB1, having hydroxyl functions at C3, C4, and C5. Analysis of the NMR data for FB2 showed very similar chemical shift values when compared to an authentic Fusarium FB2 standard, strongly indicating identical molecules despite that an absolute stereochemical assignment of FB2 from A. niger was not possible.