Although bisacetylenic oxylipins have been demonstrated to exhibit diverse biological activities, the chemical structures of many representatives of this class of phytochemicals still remain elusive. As carrots play an important role in our daily diet and are known as a source of bisacetylenes, an extract made from Daucus carota L. was screened for bisacetylenic oxylipins, and, after isolation, their structures were determined by means of LC-MS and 1D/2D NMR spectroscopy. Besides the previously reported falcarinol, falcarindiol, and falcarindiol 3-acetate, nine additional bisacetylenes were identified, among which six derivatives are reported for the first time in literature and three compounds were previously not identified in carrots. To determine the absolute stereochemistry of falcarindiol in carrots, the (3R,8S)-, (3R,8R)-, and (3S,8S)-stereoisomers of falcarindiol were synthesized according to a novel 10-step total synthesis involving a Cadiot-Chodkiewicz cross-coupling reaction of (S)- and (R)-trimethylsilanyl-4-dodecen-1-yn-3-ol and (R)- and (S)-5-bromo-1-penten-4-yn-3-ol, respectively. Comparative chiral HPLC analysis of the synthetic stereoisomers with the isolated phytochemical led to the unequivocal assignment of the (Z)-(3R,8S)-configuration for falcarindiol in carrot extracts from...