## Spontaneous substitution of azulene-derived benzylic alcohols by thiols and its application to labeling/protection of biothiols

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Azulene, a blue nonalternant aromatic compound, consists of five- and seven- membered rings of which the former has cyclopentadienyl-anion-like character. As a result, the fivemembered ring part can considerably stabilize benzylic cations. By utilizing this character, a facile benzylic substitution of azulene derivatives can be expected. Concerning this, last time, we have presented a spontaneous substitution reaction between guaiazulene-3-methanol derivatives with thiols and cysteine derivatives.

This time, we would like to expand this study to application of thiol-labeling and a protection of thiols.

Encouraged by the previous results with cysteines, along with the colored nature of azulene derivatives, the above reaction was applied to labeling of immobilized thiol-containing biomolecules. This thiol-detection was achieved as a dot-blot on the filter paper, which is convenient and not equipment-restricted. The range of biothiols covers amino acids, peptides and reduced proteins. Also, this naked-eye visualization allowed the semiquantitave analysis of glutathione, which is in the range of  $1.25 \times 10^{-4}$  mmol to  $1 \times 10^{-3}$  mmol.

From other point of view, this reaction can be regarded as a protection of a thiol. By treatment with tris(2-carboxyethyl)phosphine hydrochloride, the guaiazulene-3-ylmehyl part of the sulfide could be removed and the original thiols was recovered. It is noteworthy that the thiol can be readily isolated by a simple extraction using chloroform and water. This protective group was further applied for synthesis of cysteine derivatives and sequential deprotection of a differently protected Cys-Cys derivatives.



