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Oxidation step in the preparation of benzocamalexin: the crystallographic evidence

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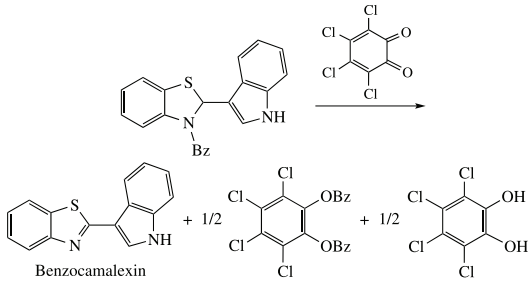
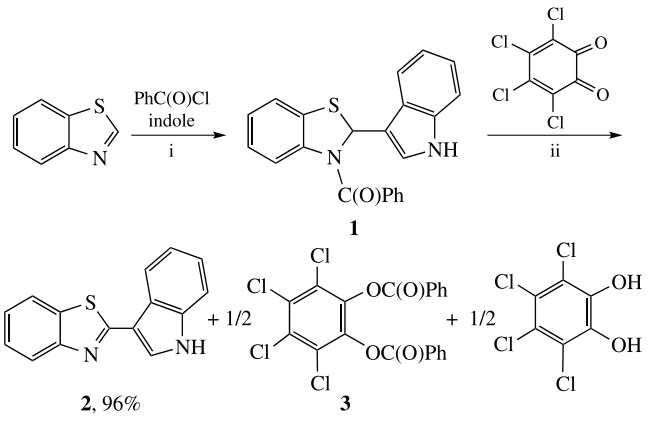
The study of oxidation step in the preparation of benzocamalexin by the α-amidoalkylation–oxidation sequence revealed the formation of perchloro-1,2-phenylene dibenzoate as the product of transformation of tetrachloro-1,2-benzoquinone applied as the oxidant. The structures of benzocamalexin and perchloro-1,2-phenylene dibenzoate were confirmed by X-ray diffraction analysis. The extraction step in the final isolation of benzocamalexin is supposed to be crucial for the complete transformation of mono- and di-acylated perchloropyrocatechol.



Keywords: benzocamalexin, phytoalexins, α-amidoalkylation, crystal structures, *o*-chloranil.

Benzocamalexin is a close analogue of camalexin, a well-studied phytoalexin[1] with important role in plant immunity and unique bioactivity profile.[2,3] The reported high cytotoxicity of benzocamalexin against a panel of human cancer cells and much lower toxicity to healthy cells[4,5] indicate a very good selectivity, which may suggest benzocamalexin as a prospective antiproliferative agent. The classical strategies to synthesize benzocamalexin are based on the condensation of 2-amino-thiophenol and indole-3-carboxaldehyde.[6–8] Recently, we proposed a new two-step synthesis of camalexin and its analogues by coupling of various aza-aromatics and indole or pyrrole as the α-amidoalkylation–oxidation sequence.[9,10] While the X-ray structure of camalexin is known since 1991,[11] there is no crystallographic data on benzocamalexin reported so far. Herein, we describe the single-crystal X-ray data of benzocamalexin and discuss the synthetic approach based on the isolated and structurally characterized secondary stoichiometric reaction product of the oxidation stage. Quinone-mediated oxidations continue to be an important synthetic tool for modification toward bioactive structures.[12] The current synthetic approach[9] is based on coupling of indole and benzothiazole in the presence of benzoyl chloride and concomitant oxidation of the obtained amidoalkylated product **1** with *o*-tetrachloroquinone (3,4,5,6-tetrachloro-1,2-benzoquinone), as depicted in Scheme 1.

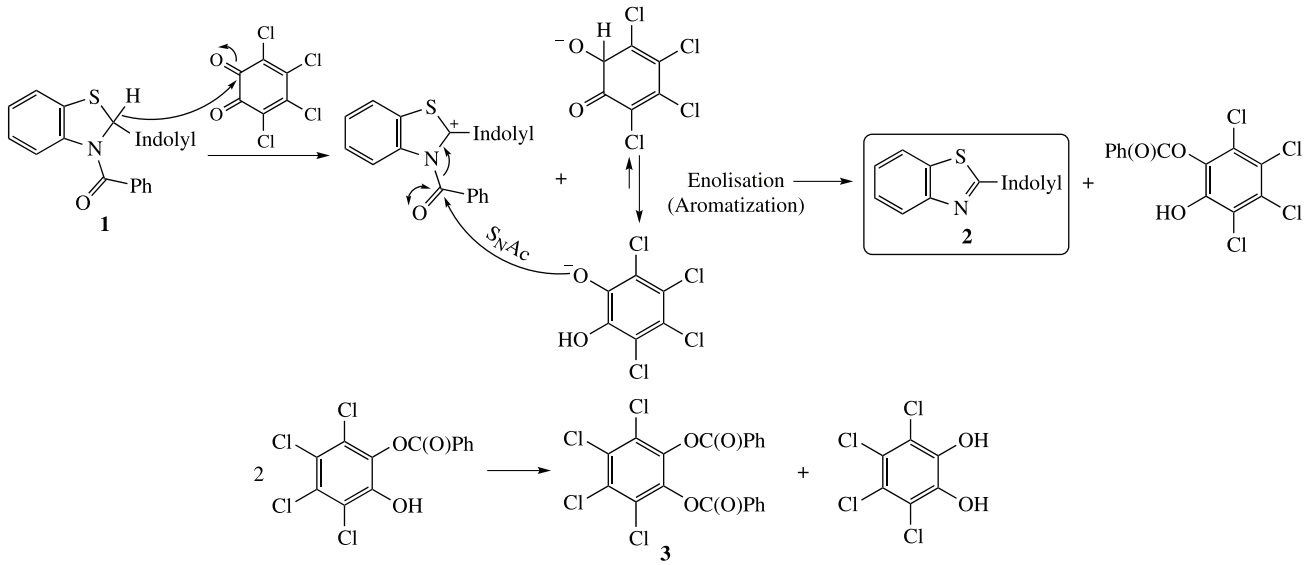


Scheme 1 *Reagents and conditions*: i, Et_3N, 1,2-dichloroethane, room temperature, 2 h; ii, *o*-tetrachloroquinone (1.5 equiv.), MeCN, 25 °C, 2 h.

The obtained compounds **1**, **2** were purified by column chromatography and characterized by ^1H, ^{13}C NMR, IR and ESI-HRMS analyses (for details, see Online Supplementary Materials, Figures S1–S9). The crystal structures of benzocamalexin **2** and oxidant transformation product **3**, perchloro-1,2-phenylene dibenzoate, were resolved by single-crystal X-ray data analysis.† Both types of crystals are colourless, and they are formed in the course of product isolation that includes extraction and column chromatography. The presence

† Colourless single crystals from compounds **2** and **3** were obtained from diethyl ether upon slow evaporation.

Crystal data for **2**. $C_{15}H_{10}N_2S$, $M = 250.31$, orthorhombic, space group *Pca*21, 120 K, $a = 24.6715(15)$, $b = 4.2684(2)$ and $c = 11.0523(5)$ Å, $Z = 4$, $V = 1163.89(10)$ Å^3, $d_{calc} = 1.428$ g cm^{-3}, $F(000) = 520$. Colourless plates with dimensions $0.23 \times 0.11 \times 0.03$ mm were analyzed on a STOE IPDS 2T 2-circle diffractometer using GeniX Cu, 0.05×0.05 mm^2 microfocus as a source for monochromated CuKα radiation ($\lambda = 1.54186$ Å) at 120(2) K. In total 1834 reflections were collected, among them 1503 unique reflections ($R_{int} = 0.0437$), and the completeness to θ was 97.1%. Final R factors: $R_1 = 0.0617$ [1503 reflections with $I > 2\sigma(I)$], $wR_2 = 0.2041$ (all reflections), GOF = 1.140.



Scheme 2

of compound **3** in the final product is not detected by NMR, IR and, however it appeared as a little co-crystallization impurity in the eluate. The resolved crystal structure of compound **3** let us propose mechanism of the oxidation step of the benzocamalexin synthesis. Our findings suggest that the solubility of non-hydrolysed dibenzoate **3** is very similar to that of benzocamalexin **2** (see Scheme 1), and the water/dichloromethane extraction step may be crucial for the complete hydrolysis of compound **3** leading to tetrachloropyrocatechol, the reduced form of *o*-tetrachloroquinone.

The kinetics of quinone-catalysed processes have revealed that the quinone function and reaction pathways directly depended on the polarity of solvent and temperature.[13–15] Triggered by these observations, we reconsidered the use of acetonitrile and mild reaction conditions to be effective for the purposes of this work. The use of DDQ (2,3-dichloro-5,6-dicyano-*p*-benzoquinone, 1.5 equiv.) as the oxidant, instead of *o*-tetrachloroquinone, led to benzocamalexin with 86% yield after chromatographic purification. In this way, no formation of O-acylated form of the reduced oxidant was observed. We assume that this is due to different polarities of DDQ and its reduced species compared to benzocamalexin, which allows for easier separation of the final product.

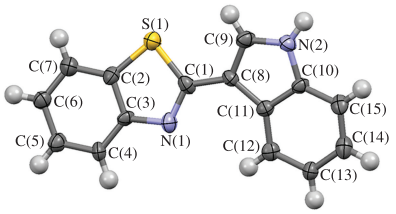


Figure 1 Structure of benzocamalexin **2** as obtained from single-crystal X-ray data (non-hydrogen atom ellipsoids are drawn at 50% probability level). Selected bond angles (°): C(2)–S(1)–C(1) 89.5(5), C(1)–N(1)–C(3) 110.6(8), C(9)–N(2)–C(10) 108.8(8), C(9)–C(8)–C(1) 122.9(9), C(8)–C(1)–S(1) 120.2(7), C(8)–C(1)–N(1) 124.6(9); and dihedral angles (°): C(9)–C(8)–C(1)–S(1) 11.3(13), C(11)–C(8)–C(1)–S(1) –173.1(8).

X-ray study of benzocamalexin **2** showed the presence of four molecules in the unit cell. The structure is almost planar with only slight tilt between the indole and the benzothiazole rings (Figure 1; for the bond lengths, see Table S1 in Online Supplementary Materials). In the crystal, benzocamalexin **2** forms a network of intermolecular hydrogen bonding that involves the N1 and N2 atoms of adjacent molecules (Figure S10). This network propagates along z axis and a two-fold screw axis of $-x,-y,1/2+z$ symmetry operator.

Several large colourless prisms (isolated from the diethyl ether eluate in the course of column chromatography) were also subjected to single-crystal X-ray analyses. The data revealed the structure of perchloro-1,2-phenylene dibenzoate **3** (Figure 2; for the bond lengths, see Table S1).

The formation of diester **3** could be explained in terms of the reaction mechanism of the oxidation step depicted in Scheme 2. The expected formation of monoacylated *o*-tetrachloropyrocatechol could proceed with its 'disproportionation' to form the isolated crystals of diacylated tetrachloropyrocatechol **3** being the oxidant transformation product along with fully reduced tetrachloropyrocatechol.

In summary, dibenzoate **3** is isolated along with the target benzocamalexin **2** when *o*-tetrachloroquinone is used as oxidant. When changing the oxidizing agent to DDQ, the product **2** is also obtained in high yield, however depriving of any impurities arising from the oxidant transformation. Apparently, this is due to different solubilities of both oxidants and/or their reduced forms in the solvents employed.

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Crystal data for **3**. $C_{20}H_{10}Cl_4O_4$, $M = 496.60$, triclinic, space group $P\bar{1}$, 120 K, $a = 8.9807(3)$, $b = 12.0300(4)$ and $c = 18.7022(6)$ Å, $\alpha = 76.971(3)$, $\beta = 86.261(3)$ and $\gamma = 79.256(3)°$, $Z = 4$, $V = 1933.45(11)$ Å^3, $d_{calc} = 1.592$ g cm^{-3}, $F(000) = 1008$. Colourless prisms with dimensions $0.27 \times 0.18 \times 0.13$ mm were analyzed on a STOE IPDS 2T 2-circle diffractometer using GeniX Mo, 0.05×0.05 mm^2 microfocus as a source for monochromated MoKα radiation ($\lambda = 0.71073$ Å) at 120(2) K. In total 3696 reflections were collected, among them 2374 unique reflections ($R_{int} = 0.0356$), and the completeness to θ was 99.2%. Final R factors: $R_1 = 0.037$ [2374 reflections with $I > 2\sigma(I)$], $wR_2 = 0.0947$ (all reflections), GOF = 1.077.

The diffraction data were corrected for absorption effects by the Gaussian integration method implemented in the STOE X-Red32 software. Unit cell parameters were calculated and refined from the full data set. The structures were solved by full-matrix least squares procedure based on F^2 using the SHELX–2014 program package[16], implemented in Olex[17] and Wingx[18] suites of programs. All non-hydrogen atoms were refined anisotropically, the positions of the hydrogen atoms were calculated using a riding model in isotropic approximation.

CCDC 2048890 and 2048889 contain the supplementary crystallographic data for compounds **2** and **3**, respectively. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* http://www.ccdc.cam.ac.uk.

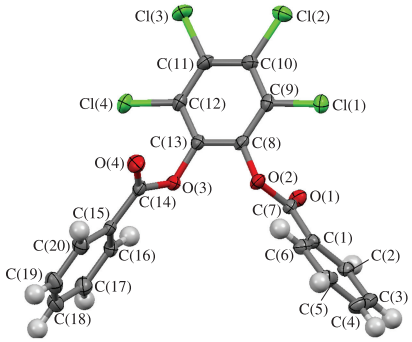

Figure 2 Structure of perchloro-1,2-phenylene dibenzoate **3** as obtained from the single-crystal X-ray data (non-hydrogen atom ellipsoids are drawn at 50% probability level). Selected bond angles (°): C(8)–O(2)–C7 115.63(16), O(1)–C(7)–C(1) 126.4(2), C(8)–C(9)–Cl(1) 118.90(16); and dihedral angles (°): C(7)–O(2)–C(8)–C13 106.5(2), O(4)–C(14)–O(3)–C(13) –11.9(3), O(1)–C(7)–O(2)–C(8) –11.4(3), C(14)–O(3)–C(13)–C8 103.7(2).

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2021.11.018.

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