

Conformational analysis of alternariol on the quantum level

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Abstract With the help of theoretical calculations we explain the phenomenon of nonplanarity of crystalline alternariol. We find out that the different orientations of the hydroxyl groups of alternariol influence its planarity and aromaticity and lead to different twists of the structure. The presence of the intramolecular hydrogen bond stabilizes the planar geometry while the loss of the bond results in a twist of over 14°. This effect is thought to be involved while cutting DNA strands by alternariol.

Keywords Alternariol · Aromaticity · Planarity · Quantum mechanics

Introduction

Alternariol (AOH, 3,7,9-trihydroxy-1-methyl-6H-dibenzo[b, d]pyran-6-one, see [1]) is a mycotoxin produced by several *Alternaria* strains, including *A. alternata*, *A. citri*, *A. solani* and *A. tenuissima* [2]. It was first described in 1953 [3] and subsequently shown to occur in a wide variety of agricultural commodities and food products like barley, wheat, apples, tomato puree, ketchup or wine [2, 4, 5]. However, only

recently an increased number of studies dealt with the toxicity of AOH in more detail. Amongst others it could be shown that AOH acts as a topoisomerase poison [6–8] thus inducing DNA strand breaks. In competitive assays AOH was also shown to bind to the minor groove of DNA [6] and to replace E2 from human oestrogen receptors α and β with its oestrogenic potential being 0.01 % that of E2 [9]. In vitro studies with porcine granulosa cells furthermore revealed that AOH inhibits the synthesis of progesterone [10], thus possibly affecting reproductive performance.

In view of the described effects, computational studies on possible toxin-receptor interactions are highly desirable. As a basis for this structural data on AOH is needed. At first glance alternariol might be seen as a conformationally rigid compound due to the completely conjugated system. It has three freely rotatable hydroxyl groups, with one of them having the possibility to form an intramolecular hydrogen bond and thus to stabilize the whole structure. The crystal structure of alternariol is published in [11]. A surprising fact is that the benzene rings of alternariol are not fully coplanar [11]. It seems that this phenomenon is due to a steric effect caused by the proximity of the 6H hydrogen to the C14 methyl group [11]. This assumption is supported by the fact that the analogue of alternariol, benzo[c]chromen-6-one-analogue-2-chloro-7-hydroxy-8-methyl-6H-benzo[c]chromen-6-one, which lacks the corresponding methyl group, is fully planar [12]. For the sum of these reasons we intended to investigate the planarity of alternariol in more detail. Another interesting aspect is the aromaticity of alternariol. It is not clear if the middle ring can be considered as aromatic and which effect the steric repulsion of the 6H hydrogen and the C14 methyl group has on the aromaticity of the ring.

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Methods

The structure of alternariol was obtained from the crystallographic study [11]. The positions of the hydroxyl groups were modified by hand with the help of Amira [13]. The geometries were optimized in the gas phase using the B3LYP-based density functional theory (DFT) approach employing the 6–311** basis set as implemented in Gaussian03 program [14]. Frequency calculations were carried out at the same level of theory in order to confirm that the optimized structures were at the minimum of the potential surface. These frequencies were then used to evaluate the zero-point vibrational energy, the thermal vibrational corrections to the enthalpy and the total enthalpy values, calculated at 273 K. The bond lengths of the optimized structures were incorporated into the harmonic oscillator model of aromaticity (HOMA) method [15] used to study aromaticity.

$$\text{HOMA} = 1 - \frac{257.7}{n} \sum_{i=1}^n (d_{\text{opt}} - d_i)^2$$

The value of 257.7 is the normalization value, n is the number of CC bonds, d_{opt} is the optimized bond length (1.388 Å) and d_i is the experimental or computed bond length.

Results and discussion

The structure of alternariol cp. Figs. 1 and 2 consists of two benzol (benzene) rings connected by a pyran ring and might therefore have a very rigid character. The only flexible part of the molecule are the three free rotatable hydroxyl groups: O3-H3, O4-H4 and O5-H5. The group O3-H3 has the possibility to build an intramolecular hydrogen bond. To explore the conformational space of the compounds, we prepared structures of the molecule with different positions of its hydroxyl groups and optimized the structures in the gas phase at B3LYP/6–311** level by using the Gaussian03 program. The optimization resulted in eight different geometries, which correspond to the dihedrals (H5 – O5 – C11 – C12) = α , (H3 – O3 – C3 – C2) = β and (H4 – O4 – C5 – C4) = γ at either near 0° or near 180°, see Figs. 3, 4 and 5 (we do not take into account the mirror geometries). Four structures have an intramolecular hydrogen bond between H3 and O2. As indicator for planarity we measured the dihedral (C13 – C8 – C7 – C6) = ϕ . Further, the energies were determined to evaluate the stability of the structures. Table 1 summarizes the results.

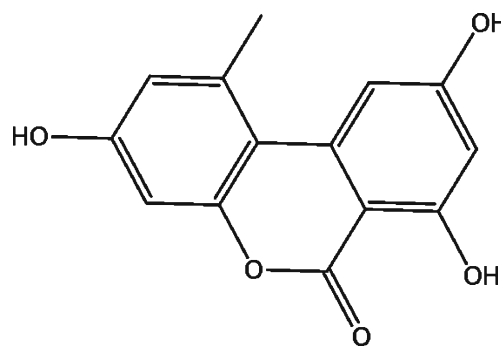


Fig. 1 Classical structural formula of alternariol

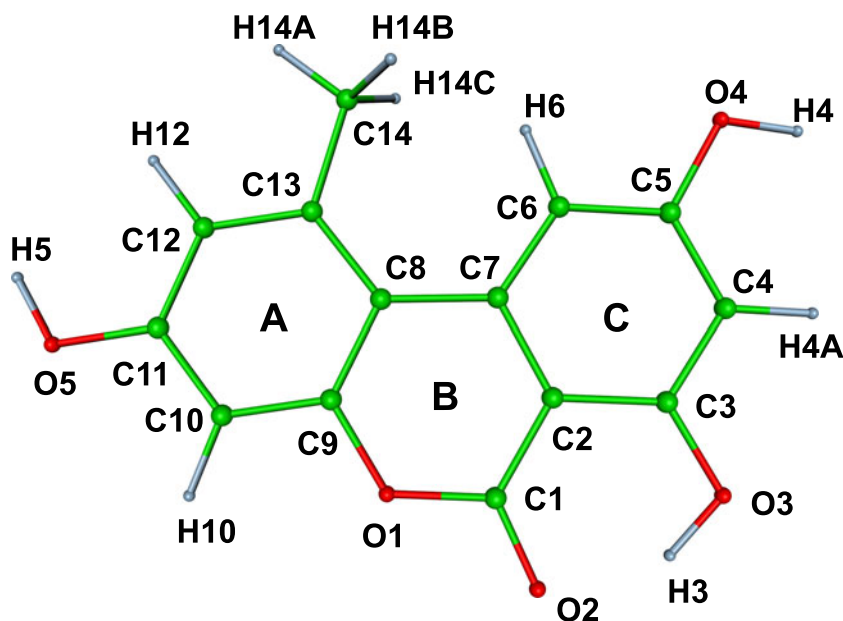
According to our studies, the energetically most favorable geometry of alternariol is indeed planar, with ϕ equal to -0.79° , α equal to -179.88° , β equal to 0.093° , γ equal to 0.046° and O3-H3 building an intramolecular hydrogen bond to O2. The optimization of the structure of alternariol obtained from the crystallographic study resulted in the second energetically most favorable geometry, showing a tight twist of ϕ being equal to -5.59° and α , β and γ being equal to 0.22° , 0.54° and 0.67° respectively. The switch of α to 179.44° and of γ to 179.89° results in the structure with a similar twist of 5.76° , which is less favorable, due to its higher relative energy of 0.8 J mol^{-1} than the most stable structure, and the switch of γ to -179.91° results in a stronger twist of -7.29° and a structure, which is energetically 1.4 J mol^{-1} less favorable than the first geometry. The four geometries without the intramolecular hydrogen bond are higher in energy by about 15 J mol^{-1} and are therefore less favorable than the structures with the bond and have a strong twist of over 14° . The strongest twist of -15.70° has the structure with α equal to 0.64° , β equal to 177.06° and γ equal to -179.67° .

Aromaticity

Aromaticity is a wide field and still an ambiguous concept. We adopt the view of a qualitative concept in which the molecules can be assigned certain specific and similar molecular properties [1]. Molecules are said to belong to this class of aromaticity if they can not be characterized by chemical bonding using one Lewis structure with two-center two-electron bonds and ion pairs and otherwise fulfill certain criteria for aromaticity. In the literature [16] many criteria for π -aromaticity are defined.

The structures with the local minima were obtained with ab initio calculations using the software code ZIBgridfree [17] and B3LYP/6–311** of the program package Gaussian03. The non-planar structures cp. Fig. 4 have a bend in the middle

Fig. 2 Amira [13]
representation of alternariol
with atomic labeling



ring. At first glance it thus seems, that alternariol is not aromatic. The celebrated Hückel rule can be used to verify, that a molecule is aromatic, however, if the Hückel rule fails, one can not suggest, that the molecule is not aromatic (e.g., coronene).

Here, we confine ourselves to three criteria for aromaticity, which we apply to alternariol.

Delocalization

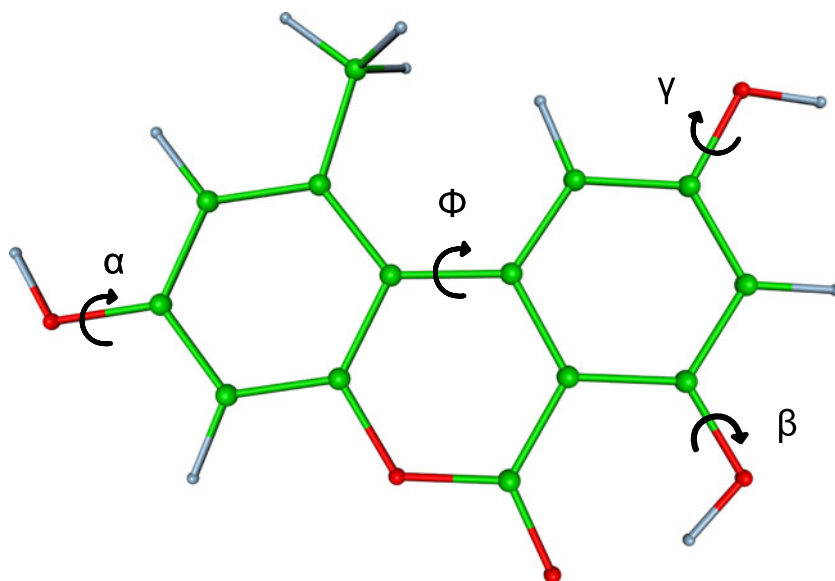
In aromatic structures the electrons show an enhanced delocalization, more precisely, the π electrons are delocated over

several C atoms (electron clouds). We therefore considered bonding character of the highest occupied molecule orbital (HOMO) for alternariol, which shows a stabilization of the first and the third cyclic ring structure. We thus identify a pronounced delocalization.

Hardness/HOMO-LUMO gap

The hardness of a molecule can be used as a measure for aromaticity. The hardness can be given by $\eta_{exact} = \left(\frac{\partial^2 E}{\partial N^2}\right)$, where E is the electronic energy and N is the number of

Fig. 3 Amira [13]
representation of alternariol
with atomic labeling and the
dihedrals α , β , γ and ϕ



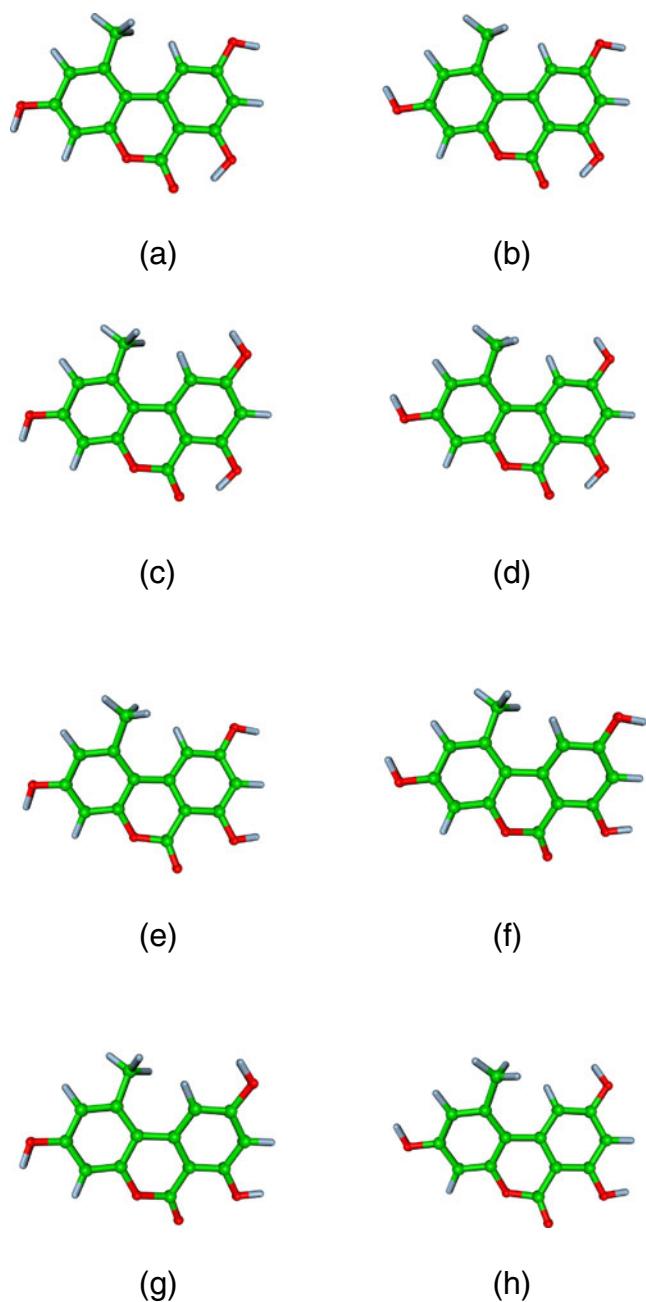


Fig. 4 Conformations of alternariol with different positions of hydroxyl groups

electrons. This can be approximated by calculating the HOMO-LUMO gap [18]. We can thus write: $\eta_{exact} \approx \eta = \frac{1}{2} (\varepsilon_{HOMO} - \varepsilon_{LUMO})$, where ε_{HOMO} and ε_{LUMO} are the energy of the HOMO and the energy of the LUMO, respectively. Molecules with large values of η indicate a high stability and are thus expected to be aromatic. In the last column of Table 2 we computed the HOMO-LUMO gap, for the different conformations. The values for η range

between -0.08185 and -0.08335 , which reveals, that alternariol has an aromatic structure [18].

HOMA

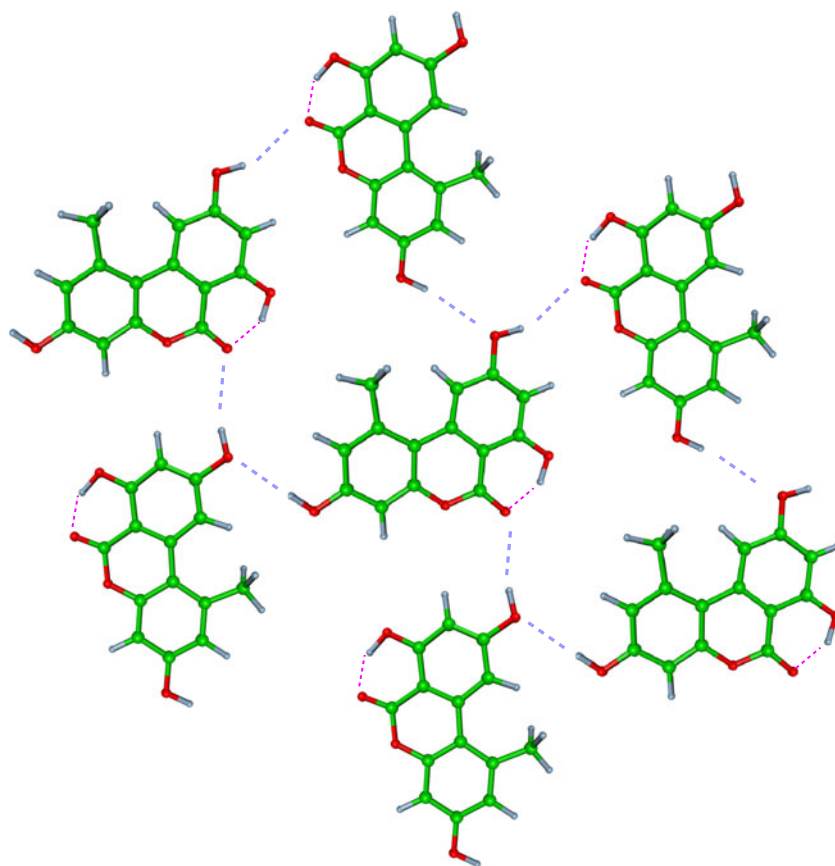
The HOMA index (harmonic oscillator model of aromatic stability) has been introduced by [15]. The HOMA index can be computed by $HOMA = 1 - \frac{a}{n} \sum_{i=1}^n (d_{opt} - d_i)^2$, where d_{opt} is the optimal bond length, d_i is the length of bond i , n is the number of bonds and a is an empirical constant given by $a=257.7$. The HOMA index gives a measure for the similarity of the length of the bonds in a molecule. The closer the value is to one, the more equal the bonds and thus the more aromatic the molecule. The HOMA index can also be applied to polycyclic systems like alternariol. In Table 2 we computed the HOMA index for each ring and each conformation. It can be seen, that ring A and ring C show values close to one, and are thus expected to be aromatic. However the values for ring B do not suggest an aromatic structure.

Summing up, we have applied three criteria in order to decide, whether alternariol is aromatic or not. We remark that all these indicators for aromaticity have to be taken with care. For instance, according to the η from the HOMO-LUMO gap indicator, ethene should be aromatic, which is not true. We thus carefully draw the deduction, that alternariol shows a tendency to an aromatic structure, which is supported by the aromatic structure of ring A and C and detained by ring B.

Conclusions

Our results show that the planarity of alternariol is dependent on the orientations of its three free rotatable hydroxyl groups. The most stable geometry of alternariol is planar. It has an intramolecular hydrogen bond between O3-H3 and O2, which stabilizes the structure. The orientations of O4-H4 and O5-H5 result in the farthest possible positions of H5 to H12 and of H4 to H6. Thus, the steric effect caused by the proximity of the H6A hydrogen to the C14 methyl group lead to increases of C14-C13-C8 without destroying the planarity of the molecule. The change of orientations of O4-H4 and O5-H5 results in the placement of H5 close to H12 and H4 to H6. Due to steric effects, the molecule undergoes a tight twist to keep H5, H12, C14 methyl group, H6A and H4 as far away from each other as possible. Geometries of alternariol without the intramolecular hydrogen bond between O3-H3 and O2 are less stable than geometries with the intramolecular hydrogen bond, but these structures could play a role if alternariol is placed in the

Fig. 5 Schematic representation of crystalline alternariol with hydrogen bonds



vicinity of other molecules with polar groups. Building of intermolecular hydrogen bonds between alternariol and other molecules could then result in a twist of alternariol. It is possible that this effect is involved while cutting DNA strands by alternariol. Crystalline alternariol consists of molecules with the geometry corresponding to Fig. 4 (b). However, the presence of the intramolecular hydrogen bond between O5-H5 of one molecule and O4 of the neighbor

molecule results in an alternariol conformation which can be seen as a mixture of Figs. 4 (b) and (d). Thus, crystalline alternariol shows a twist of circa 7° .

Table 1 Dihedral values of geometries which correspond to local minima of the energy landscape of alternariol and relative energies ϵ in J mol^{-1}

Conformation	α	β	γ	ϕ	ϵ
(a)	179.88	-0.093	-0.046	0.79	0.0
(b)	-0.22	-0.54	-0.67	5.59	0.4
(c)	179.44	-0.56	179.89	5.76	0.8
(d)	-0.33	-0.71	179.91	7.29	1.4
(e)	178.82	-177.05	-1.50	14.42	15.6
(f)	-0.73	-176.90	-1.52	14.94	16.2
(g)	178.70	-177.16	179.57	15.20	15.3
(h)	-0.64	-177.06	179.67	15.70	15.9

Table 2 HOMA is evaluated for the three six-membered rings in alternariol. HOMA would be zero for a nonaromatic system and one for a system with all bonds equal to an optimal value d_{op} . It is believed to indicate aromaticity when it is close to one. HOMA predict that the left and the right rings have comparable high aromaticities whereas the middle ring is more nonaromatic. The last column shows energy value of the highest occupied molecular orbital minus the lowest unoccupied molecular orbital in atomic units (a.u.)

Conformation	HOMA ring A	HOMA ring B	HOMA ring C	Gap HOMO-LUMO
(a)	0.894	0.451	0.853	-0.1641
(b)	0.894	0.451	0.868	-0.1637
(c)	0.894	0.451	0.859	-0.1637
(d)	0.894	0.451	0.859	-0.1640
(e)	0.894	0.324	0.874	-0.1660
(f)	0.894	0.324	0.874	-0.1667
(g)	0.894	0.324	0.905	-0.1660
(h)	0.926	0.324	0.905	-0.1666

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