



## Structural and electronic properties of ajoene molecule

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### Abstract

The structural and electronic properties of ajoene molecule have been investigated theoretically by performing semi-empirical molecular orbital theory calculations. The geometry of the system has been optimized and the electronic properties of the system considered has been calculated by semiempirical self-consistent-field molecular orbital theory at the AM1 level within RHF formalism in its ground state.

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### 1. Introduction

Garlic (*Allium sativum* L., Liliaceae) contains more than 100 biologically useful secondary metabolites. Besides alliinase, allicin, S-allylcystein, diallylsulfide and allymethyltrisulfide; alliin is present as the major sulfur-containing compound. When raw garlic bulb is crushed/damaged, alliinase is released from the tissue and produces allicin by cleaving alliin from garlic oil [1]. It is an important substance for the medicinal properties of garlic. Allicin is in turn the precursor to several sulfur-containing compounds responsible for the flavor, odor and pharmacological properties of garlic and is rapidly converted to diallyldisulfide and others because of its instability. *Allium* spp. has also been reported to contain high

levels of potent antioxidant quercetin and its derivatives. Potential antioxidant activity aids in the protection against oxidative stress related diseases. Some of these effects are synergistic to nitric oxide. Ajoene, one of the derivatives of allicin, has been found as a major sulfur-containing compound in an oil-macerated garlic extract and it has been described essentially as a potent inhibitor of platelet aggregation in vitro and in vivo. It is formed by self-condensation from allicin but is not present in dehydrated garlic powder [2,3].<sup>1–3</sup>

(*E,Z*)-ajoene ([92285-01-3] 2-propenyl 3-(2-propenylsulfanyl)-1-propenyl disulfide; 4,5,9-trithiadio-deca-1,6,11-triene 9-oxide; C<sub>9</sub>H<sub>14</sub>OS<sub>3</sub>) has *cis*-(*Z*-) and *trans*-(*E*-) isomers, both with varying degrees

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<sup>1</sup> <http://www.umt.edu/medchem/teaching/medchem/mclect1.htm>

<sup>2</sup> <http://www.microscopy.fus.edu/phytochemicals/pages/ajoene.html>

<sup>3</sup> <http://wilkes1.wilkes.edu/~kklemow/Allium.html>

and specificities of bioactivity. The diastereomer with Z configuration being slightly more potent [2]. Ajoene is only found in small quantities in natural garlic oil. Z-ajoene arrested HL60 cells in G(2)M phase of cell cycle in a dose and time dependent way. In PtK2 cell, it induced a complete disassembly of the microtubule network, that was associated with an increased number of cells blocked in early mitotic stages. Z-ajoene inhibited tumor growth by 38% and 42% in mice grafted with sarcoma 180 and hepatocarcinoma 22, respectively. The microtubule cytoskeleton appeared to be one of the Z-ajoene targets. The ability of Z-ajoene to preferentially suppress the growth of neoplastic cells could provide a new approach in tumor therapy [4–8].

Ajoene has been shown to induce apoptosis in a leukemic cell line as well as blood cells of leukemic patient. Activation of caspases was necessary for ajoene-induced apoptosis. Ajoene induced the release of cytochrome *c*. It also led to a dissipation of the mitochondrial transmembrane potential [9]. The interactions between the flavoenzymes and ajoene are expected to increase the oxidative stress of the respective cell [10]. Ajoene has been shown to be effective in short-term treatment of tinea pedis [11]. The parent compound allicin is also a powerful antifungal agent.

Ajoene protected acutely infected Molt-4 cells against HIV-1 and blocked further destruction of CD4-T cells in vitro. The mode of anti-HIV action of ajoene can be ascribed to the inhibition of early events of viral replication, particularly virus adsorption [12].

Ajoene has also been shown to reduce nitrite accumulation, a parameter for nitric oxide synthesis, in lipopolysaccharide (LPS)-activated RAW 264.7 macrophages dose indicating that allicin and ajoene inhibit the expression of iNOS (inducible NO synthase) in activated macrophages. There was a reduction in iNOS mRNA steady-state levels [13]. Ajoene interaction with model membranes revealed significant modifications in phospholipid dynamics using ESR experiments. The interaction resulted in an increase of the membrane fluidity in its hydrophobic part and could be related to clinical properties of ajoene [14].

Ajoene impairs platelet aggregation by inhibiting the functional exposure of platelet integrins GPIIb/IIIa. Strong inhibition of the proliferation induced human lymphocytes by the mitogens

phytohemagglutinin (PHA), phorbol myristate acetate (PMA) and anti CD-3, and the capping formation induced in B lymphocytes by anti-IgM antibodies were reported for ajoene. On macrophages, ajoene was also found to partially inhibit the LPS-induced production of tumor necrosis factor (TNF) and decrease the phagocytic activity of thioglycolate-elicited mouse peritoneal macrophages for IgG-opsinized human erythrocytes. Ajoene is a potent modulator of membrane-dependent functions of immune cells [15].

Ajoene has also been reported to exhibit antimutagenic and antibiotic effects such as antimicrobial, antiviral and antiprotozoal activity. Inactivation of superoxide radicals by presence of garlic extract leads to the decrease of mutagenic effects of adriamycin. However, garlic extract does not stimulate DNA-repair systems [16–19]. Polasa and Krishnaswamy [20] reported a significant reduction in the excretion of urinary mutagens by carcinogen-exposed rats fed dried powdered garlic. Work continues in the search for therapeutically useful organosulfur compounds similar to ajoene in oil-macerated garlic products. Recently, Calvey et al. [21,22] isolated and identified cepaenes, structurally related compounds to ajoene, both from ramp (*Allium tricoccum*, wild leek, closely related plant to wild garlic species) and onion, and reported antithrombotic activity.

Similar to ajoene, *E*-10-devinylajoene (iso-*E*-10-DA), another oil-macerated garlic extract compound, had antimicrobial activity against Gram positive bacteria. Trans-structure and/or the position of double bond of iso-*E*-10-DA reduces the antimicrobial activity [18]. Two other antioxidants isolated and identified from garlic shoot were kaempferol-3-*O*-beta-D-glucopyranose and isorhamnetin-3-*O*-beta-D-glucopyranose [23].

Antithrombotic activity is associated with disulfides directly attached to a phenyl ring and is further enhanced by an alpha-sulfonyl group. CH<sub>3</sub>SO<sub>2</sub>CH<sub>2</sub>-SSPh proved to be a potent inhibitor of platelet aggregation, with an IC<sub>50</sub> of 5 μM [3]. Dirsch and Vollmar [24] have shown ajoene to dose-dependently inhibit the release of LPS-induced prostaglandin E<sub>2</sub> in RAW 264.7 macrophages. This effect was attributed to an inhibition of cyclooxygenase enzyme activity by ajoene. Ajoene was proposed to be a non-steroidal anti-inflammatory natural product.

This is the first report on the theoretical investigation of ajoene, the thermally labile organosulfur compound from garlic, because of its biological and medical importance. The results of such theoretical work will aid in the elucidation of structure–activity relationships of anticoagulant activity of compounds in garlic extract before they can be safely evaluated and commercially developed as beneficial pharmaceuticals for the treatment of blood clotting disorders and reducing the risk of stroke and cardiovascular disease.

## 2. Method of calculation

In the present study, the ajoene molecule has been considered theoretically for the first time by performing semi-empirical molecular orbital theory calculations in the gas phase. Preoptimization has been performed by applying the molecular-mechanics method [25] using MM + force field [26]; this makes it easier to perform full optimization by extended methods. The Austin Model 1 (AM1) semi-empirical method [27] within the Restricted Hartree–Fock (RHF) [28] formalism has been considered to optimize fully the geometry of the system considered. Geometry optimization is carried out by using a conjugate gradient method (Polak–Ribiere algorithm [29]), then the electronic structure of the system has been calculated by applying the semiempirical self-consistent-field molecular orbital (SCF MO) method within the RHF formalism. The SCF convergency is set to  $0.001 \text{ kcal mol}^{-1}$  and the RMS gradient is set to  $0.001 \text{ kcal Å mol}^{-1}$  in the calculations. We have performed all the calculations by using the HyperChem-7 packet [30].

## 3. Results and discussion

The closed formula of the ajoene molecule is in the form  $\text{C}_9\text{H}_{14}\text{OS}_3$ . AM1 method considers 74 valence electrons in ajoene molecule; there are 37 doubly occupied levels. Sixty six molecular orbitals have been considered in the calculations. Ajoene molecule has singlet multiplicity in its ground state, the molecular point group of ajoene is  $\text{C}_1$ . Ajoene molecule has two isomers in garlic-oil extracts; *Z*-ajoene in *cis*-form and

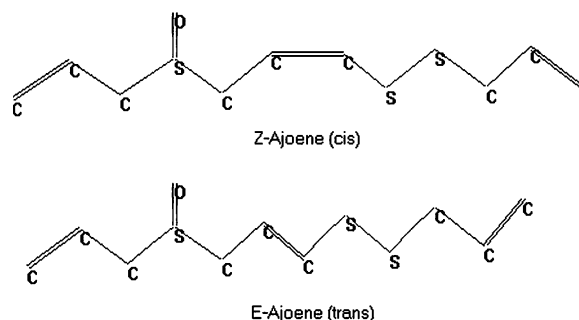


Fig. 1. Naturally occurring ajoene isomers in garlic-oil extracts [5].

*E*-ajoene in *trans*-form. Naturally occurring ajoene isomers in garlic-oil extracts are shown schematically in Fig. 1. The geometry optimization of AM1 method yields a nonplanar and nonlinear structure as the stable form of isolated ajoene molecule in gas phase. Interestingly here we show for the first time that both ajoene isomers have the same geometry after optimization in the gas phase. The optimized geometry, atom labels, and the excess charge on the atoms of the ajoene molecule are shown in Fig. 2. The bond lengths of the optimized ajoene molecule are shown in Fig. 3. The molecular orbital eigenvalue spectra of the ajoene molecule is given in Fig. 4. The 3D plots of the HOMO

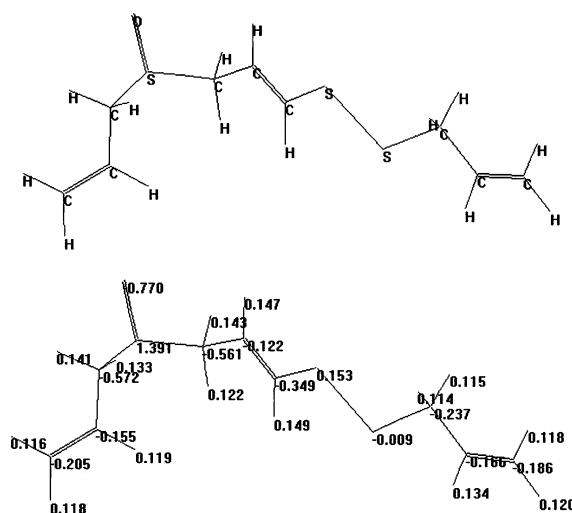


Fig. 2. The optimized structure of the ajoene molecule. Both isomers have the same structure after optimization in the gas phase. The structure of ajoene is nonplanar and nonlinear with  $\text{C}_1$  symmetry in its ground state; optimization has been performed by AM1 method. Calculated excess charge on the atoms of ajoene are given on the lower panel.

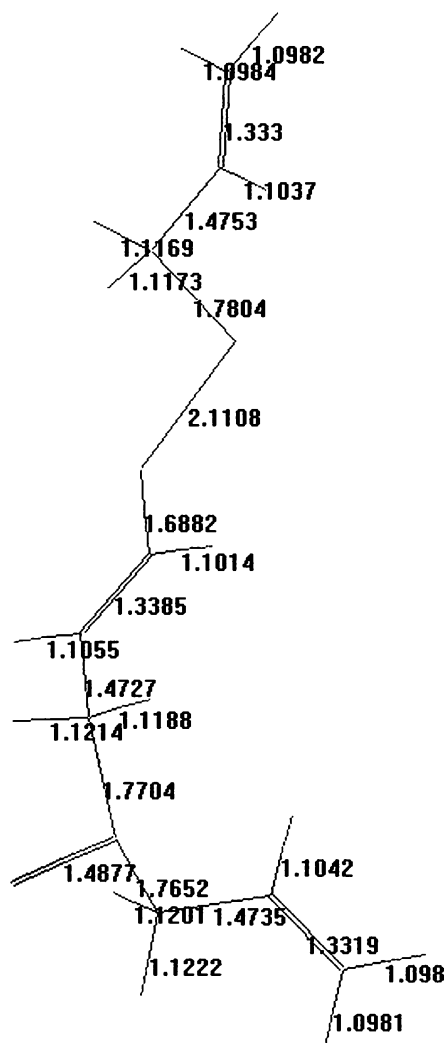


Fig. 3. The bond lengths of the optimized structure of the ajoene molecule.

and LUMO of the ajoene molecule are shown in Fig. 5. The 3D plots of the total charge density and the electrostatic potential of the ajoene molecule are shown in Fig. 6.

There is a large positive charge development (about  $+1.4e$ ) on the sulfur atom bonded to oxygen, other two sulfur atoms have relatively less excess charge. Oxygen atom has negative excess charge of about  $-0.8e$ , which is the only atom in ajoene molecule having attractive potential. Oxygen atom in ajoene may play an important role in the interaction of



Fig. 4. The molecular orbital eigenvalue spectra of the ajoene molecule.

ajoene and its environment. The highest occupied molecular orbitals are localized mainly on the carbon atoms having double bond in the central part of the molecule, however, the lowest unoccupied molecular orbitals are localized mainly on the sulfur atoms bonded to each other. Hydrogen atoms bonded to central double bonded carbon atoms have the largest positive excess charge (about  $+0.15e$ ) among all the hydrogen atoms in the molecule; these hydrogen atoms are expected to leave the molecule first in a proton transfer reaction. This may be related to potential antioxidant activity of ajoene.

The calculated energy values of the system studied are given in Table 1. The highest level energy,

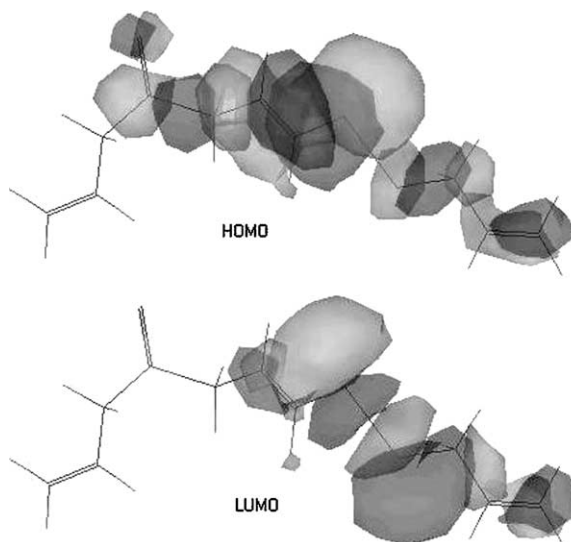


Fig. 5. 3D plots of HOMO and LUMO of ajoene molecule.

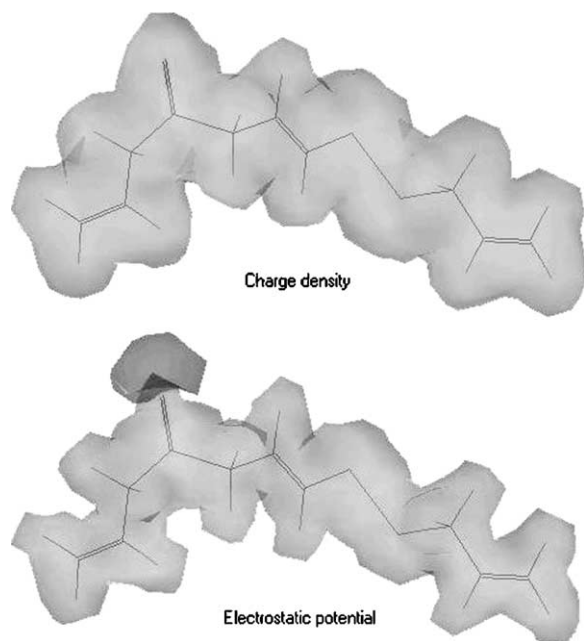


Fig. 6. 3D plots of total charge density and electrostatic potential of the ajoene molecule.

the highest occupied and the lowest unoccupied molecular orbital energies (HOMO and LUMO, respectively), the frontier molecular orbital energy gap (LUMO–HOMO energy difference,  $E_g$ ), the lowest level energy, and the calculated dipole moment values of the system considered are also given in Table 1.

According to the AM1 calculation binding energy of the ajoene molecule is about  $-2511 \text{ kcal mol}^{-1}$ ,

Table 1

Some of the calculated energy values and dipole moment ( $\mu$ ) of the ajoene molecule in its ground state. TE: total energy; BE: binding energy; IAE: isolated atomic energy; EE: electronic energy; CCI: core–core interaction; HoF: heat of formation; LMO: lowest molecular orbital; HOMO: highest occupied molecular orbital; LUMO: lowest unoccupied molecular orbital;  $E_g$ : HOMO–LUMO difference (frontier molecular orbital energy gap)

Quantity(kcal/mol)	Value	Quantity	Value
TE	-51820.053	LMO (eV)	-36.109
BE	-2511.392	HOMO (eV)	-8.747
IAE	-49308.661	LUMO (eV)	-1.840
EE	-265009.204	$E_g$ (eV)	6.907
CCI	213189.151	HMO (eV)	5.378
HoF	14.805	$\mu$ (Debye)	3.944

heat of formation of ajoene is about  $15 \text{ kcal mol}^{-1}$  and it is endothermic. Frontier molecular orbital energy gap, namely the HOMO–LUMO gap,  $E_g$  of the ajoene molecule is about 7 eV. The ajoene molecule has a dipole moment of about 4 Debyes. This dipole moment value is not small, therefore ajoene molecule may be considered to be a sort of polar molecule and interacts with its aqueous environment easily.

Theoretical investigations will aid in the elucidation of antiplatelet aggregation and antioxidant activity and clarification of the uncertainty about the conditions under which biologically active molecules such as ajoene or other potential health hazards before they can be safely evaluated and commercially developed as beneficial therapeutic agents.

## References

- [1] E.B. Kuettner, R. Hilgenfeld, M.S. Weiss, Arch. Biochem. Biophys. 402 (2002) 192–200.
- [2] E. Block, Sci. Am. 252 (1985) 94–99.
- [3] J.A. MacDonald, R.F. Langler, Biochem. Biophys. Res. Commun. 273 (2002) 421–424.
- [4] M. Li, J.-R. Ciu, Y. Ye, L.-H. Zhang, K. Wang, M. Gares, J. Cross, M. Wright, J. Leung-Tack, Carcinogenesis 23 (2002) 573–579.
- [5] M.J. Wargovich, Dis. Colon Rectum 31 (1988) 72–75.
- [6] S. Belman, J. Solomon, A. Segal, E. Block, G. Barany, J. Biochem. Toxicol. 4 (1989) 151–160.
- [7] Y. Dong, D. Lisk, E. Block, C. Ip, Cancer Res. 61 (2001) 2923–2928.
- [8] R.G. Mehta, V. Steele, G.J. Kelloff, R.C. Moon, Anticancer Res. 11 (1991) 587–591.
- [9] V.M. Dirsch, D.S.M. Antlsperger, H. Hentze, A.M. Vollmar, Leukemia 16 (2002) 74–83.
- [10] H. Gallwitz, S. Bonse, A. Martinez-Cruz, I. Schlichting, K. Schumacher, R.L. Krauth-Siegel, J. Med. Chem. 42 (1999) 34–372.
- [11] E. Ledezma, K. Marcano, A. Jorquera, L. DeSousa, M. Padilla, M. Pulgar, J. Amer. Acad. Dermatol. 43 (2000) 829–832.
- [12] R. Walder, Z. Kalvatchev, D. Garzaro, M. Barrios, R. Apitz-Castro, Biomed. Pharmacother. 51 (1997) 379–403.
- [13] V.M. Dirsch, K.K. Alexandra, H. Wagner, A.M. Vollmar, Atherosclerosis 139 (1998) 333–339.
- [14] J.C. Debouzy, J.M. Neumann, M. Herve, D. Daveloose, J. Viret, R. Apitz-Castro, Eur. Biophys. J. 17 (1989) 211–216.
- [15] E.L. Romano, R.F. Montano, B. Brito, R. Apitz, J. Alonso, M. Romano, S. Gebran, A. Soyazo, Immunopharmacol. Immunotoxicol. 19 (1997) 15–36.

- [16] K. Ishikawa, R. Naganaura, H. Yoshida, N. Iwata, H. Fukuda, T. Fujino, A. Suzuki, *Bioscience, Biotech. Biochem.* 60 (1996) 2086–2088.
- [17] S. Knasmüller, R. de Yoshida, G. Domjan, A. Szakmary, *Environ. Mol. Mutagen.* 13 (1989) 357–365.
- [18] H. Yoshida, H. Katzuzaki, R. Ohta, K. Ishikawa, H. Fukuda, T. Fujino, A. Suzuki, *Bioscience, Biotech. Biochem.* 63 (1999) 588–590.
- [19] E. Ledezma, A. Jorquera, H. Bendezu, J. Vivas, G. Perez, *Parasitol. Res.* 88 (2002) 748–753.
- [20] K. Polasa, K. Krishaswamy, *Cancer Lett.* 114 (1997) 185–186.
- [21] E.M. Calvey, J.E. Matusik, K.D. White, R. DeOrazio, D. Sha, E. Block, *J. Agric. Food Chem.* 45 (1997) 4406–4413.
- [22] E.M. Calvey, K.D. White, J.E. Matusik, D. Sha, E. Block, *Phytochemistry* 49 (1998) 359–364.
- [23] M.-Y. Kim, S.-W. Choi, S.-K. Chung, *Food Sci. Biotechnol.* 9 (2000) 199–203.
- [24] V.M. Dirsch, A.M. Vollmar, *Biochem. Pharmacol.* 61 (2001) 587–593.
- [25] U. Burkert, N.L. Allinger, *Molecular Mechanics*, ACS Monograph 177 (2001) 1982.
- [26] N.L. Allinger, *J. Am. Chem. Soc.* 99 (1977) 8127–8134.
- [27] J.J.P. Stewart, *J. Comp. Aided Molec. Design* 4 (1985) 1–108.
- [28] C.C.J. Roothaan, *Rev. Mod. Phys.* 23 (1951) 69–103.
- [29] P. Fletcher, *Practical Methods of Optimization*, Wiley, New York, 1990.
- [30] Hypercube, Inc., Gainesville, FL, USA.