Toward a Rationalization of the Sensitizing Potency of Substituted *p*-Benzoquinones: Reaction of Nucleophiles with *p*-Benzoquinones

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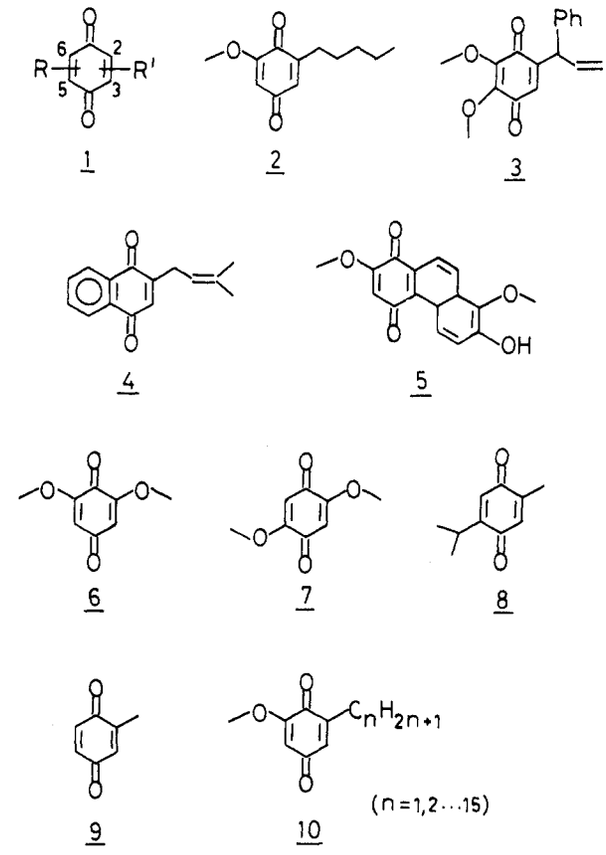
MNDO calculations have been carried out for the contact sensitizers 2,6-dimethoxy-1,4-benzoquinone (**6**) and 2-methoxy-6-methyl-1,4-benzoquinone (**10**) and for 2,5-dimethoxy-1,4-benzoquinone (**7**), which is nonallergenic in contrast to thymoquinone (**8**) (2-methyl-5-isopropyl-1,4-benzoquinone), which is a relatively strong contact allergen. Theoretical results indicate that the conformational flexibility of methoxy groups substituted at the quinone rings influences the electronic properties of these compounds, in particular their reactivity with regard to nucleophiles. According to theory, **6**, **10**, and **8** should possess a pronounced reactivity toward nucleophiles while **7** should resist nucleophilic attack. Hence, the allergenic capacity of a quinone seems to depend on their binding interactions with nucleophiles such as amino or thio groups of amino acids.

Quinonoid compounds, in particular substituted *p*-benzoquinones (**1**, Scheme I), have been known as contact allergens for a long time.[1] All the experimental evidence gathered up to now suggests that quinones substituted completely at their ring positions show no sensitizing potency while those quinones with one or two free ring positions exhibit a more or less strong sensitizing capacity.[2,3] Experiments confirm that a structural entity O=C—C=CHR in low-molecular-weight compounds can be attacked by nucleophiles to yield covalently bonded adducts.[4-6] In the skin, the nucleophiles are probably functional groups of amino acids of receptor proteins, e.g., SH of cysteine or NH_2 of lysine. The assumption that sensitizing capacity is a result of a nucleophilic attack on the entity O=C—C=CHR rationalizes the allergenic properties of quinonoid compounds as well as sesquiterpene lactones (Figure 1).

Quinonoid sensitizers belong to the strongest contact allergens found in nature. Among the more important ones are primin (2-methoxy-6-pentyl-1,4-benzoquinone) (**2**) from *Primula* species and (*R*)-3,4-dimethoxydalbergione (2,3-dimethoxy-5-(1-phenylallyl)-1,4-benzoquinone) (**3**) from the heartwood of the Brazilian pao ferro (*Machaerium scleroxylum* Tul.). A representative of the allergenic naphthoquinones is deoxylapachol (2-(γ,γ-dimethylallyl)-1,4-naphthoquinone) (**4**) from *Tectona grandis* L. (teakwood), while lapachole, substituted at C3 with a hydroxy group, is not a sensitizer. An example for phenanthrenequinones is cypripedin (7-hydroxy-2,8-dimethoxy-1,4-phenanthrenequinone) (**5**) from the orchid *Cypripedium calceolus* L. (lady's slipper). All these allergens exhibit allergenic cross reactivities, which is in accordance with their structural similarities at the quinonoid ring system.[7] The space filled by the superimposed geometries of **2**, **3**, **4**, and **5** shown in the stereoplot of figure 4 is sufficient to embed all structures given in Scheme I (**10** up to $n = 5$). Furthermore, it allows rotation of methoxy groups located at positions 2, 5, and 6 of the quinone ring (see **6** and **7** in Scheme I).

A weak to moderate sensitizer occurring in more than 50 plant species is 2,6-dimethoxy-1,4-benzoquinone (**6**)[3] while the congener 2,5-dimethoxy-1,4-benzoquinone (**7**) is nonallergenic.[8] On the other hand, it is known that the 2,5-disubstituted thymoquinone (2-methyl-5-isopropyl-1,4-benzoquinone) (**8**) from cedar wood is a relatively strong sensitizer,[8] and the simple toluquinone **9** (2-methyl-1,4-benzoquinone) is allergenic as well.[9] The synthetic 2-methoxy-6-methyl-1,4-benzoquinone (**10**, $n = 1$) is a weak contact sensitizer similar to **6**.

Scheme I. Quinonoid Contact Sensitizers[a]



[a] Compound **7** is nonallergenic.

In order to rationalize these observations, in particular the striking differences in the sensitizing properties of **6**,

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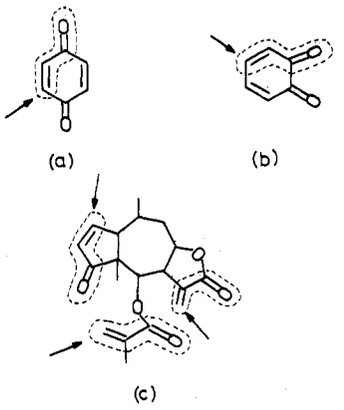

Figure 1. *p*-Benzoquinone (a), *o*-benzoquinone (b), and sesquiterpene lactone (c) containing the entity CH=CH—C=O (with in dashed lines). Positions of a possible attack by a nucleophile are indicated by arrows.

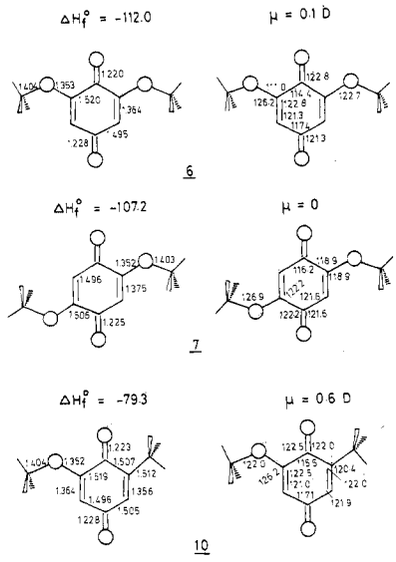

Figure 2. Calculated heats of formation $\Delta H_f°$ (in kilocalories/mole), geometries (distances in angstroms, angles in degrees), and dipole moments μ (in debye).

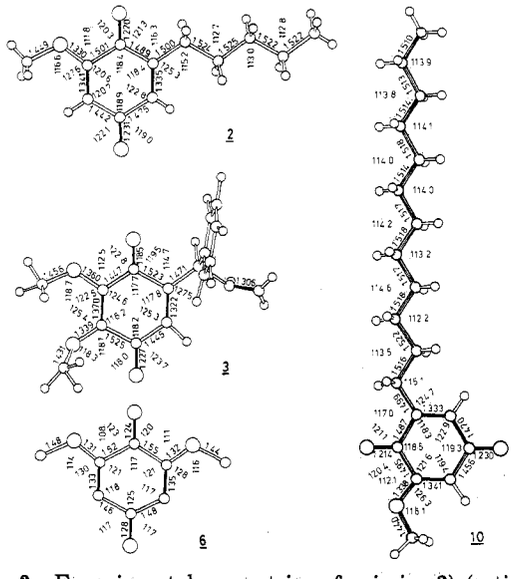

Figure 3. Experimental geometries of primin (**2**) (estimated standard deviations, 0.004–0.006 Å and 0.3° for all angles), (*R*)-3,4-dimethoxydalbergione (**3**) (esd's, 0.010–0.018 Å and 1.0–1.2°), 2,6-dimethoxy-1,4-benzoquinone (**6**) (esd's, 0.02 Å and 1.0–2.0°), and 2-methoxy-6-dodecyl-1,4-benzoquinone (**10**) (esd's, 0.003–0.005 Å and 0.3–0.5°).

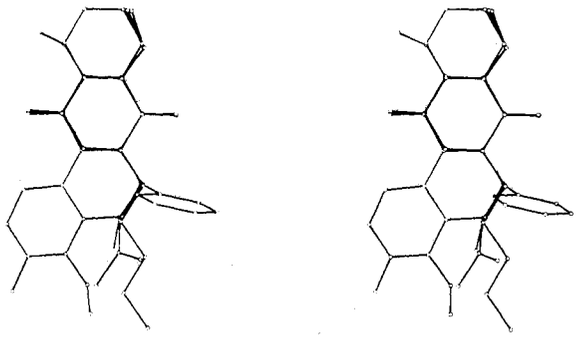

Figure 4. Superimposed structures of primin (**2**), (*R*)-3,4-dimethoxydalbergione (**3**), deoxylapachol (**4**), and cypripedin (**5**).

7, and **8**, we have investigated the reactivity of substituted *p*-benzoquinones with regard to nucleophiles by theoretical means. For this purpose, we carried out quantum chemical calculations for **1** with R (R′) = H, CH_3, or OCH_3 by employing the semiempirical MNDO method,[10] which is suitable for calculations on relatively large molecules. Various conformations of methoxy- and methyl-substituted **1** were considered by keeping dihedral angles τ(C1C2OC), τ(C1C6OC), and τ(C1C6CH), respectively, at predetermined values (0°, 90°, 180°) and optimizing all other geometrical parameters of **1**. Analyzing the results of these calculations, we assessed conformational (steric) and electronic properties of **1**, which may influence its reactivity in the presence of nucleophiles. We have exclusively considered nucleophilic attack on those ring positions in **1** that do not bear a substituent R. Certainly, a nucleophile may also attack at other ring positions of **1**, including the carbonyl C atoms. However, these reactions seem to be of no relevance in the present context since an allergen belonging to the class of 2,3,5,6-tetrasubstituted **1** has not been observed.

Results and Discussion

In Figure 2, heats of formation $\Delta H_f°$ and relevant geometrical data of **6**, **7**, and **10** (n = 1) are summarized. For the purpose of comparison, experimentally determined geometries[11-14] of **2**, **3**, **6**, and **10** are shown in Figure 3. Considering the different substitution patterns, theoretical and experimental geometries are in reasonable agreement. This suggests that the MNDO approach is appropriate for the problems considered.

The effect of a methoxy substituent on the reactivity of **1** is both steric and electronic. For example, in the most stable conformation of **6** (**6a** in Figure 5), the methyl groups partially shield positions 3 and 5, thus impeding nucleophilic attack at these positions. However, rotation at the bond C2–O (C6–O) is a low-energy process as revealed by theory. A conformational change of **6a** to **6b** (Figure 5) requires just 8 kcal/mol. Hydrophobic interactions between phenyl or alkyl groups of the receptor protein and the methyl groups of **6** may be sufficient to stabilize **6b** relative to **6a**. Rotation of the methoxy groups

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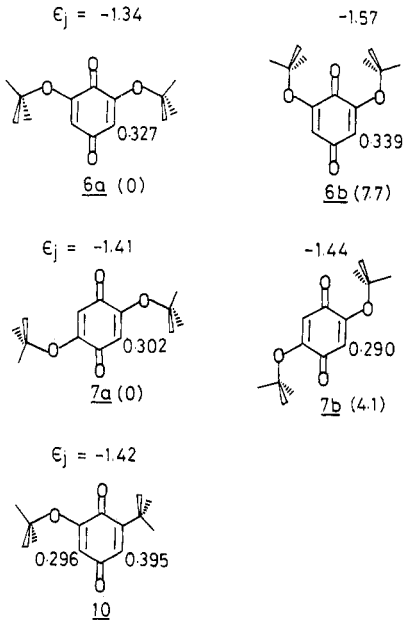

Figure 5. Conformations **a** and **b** of *p*-benzoquinones **6** and **7**. Calculated LUMO energies ϵ_j (in electronvolts) and LUMO coefficients c_{ja} (only for a = 3 and/or 5) are given. Numbers in parentheses denote relative enegies (in kilocalories/mole).

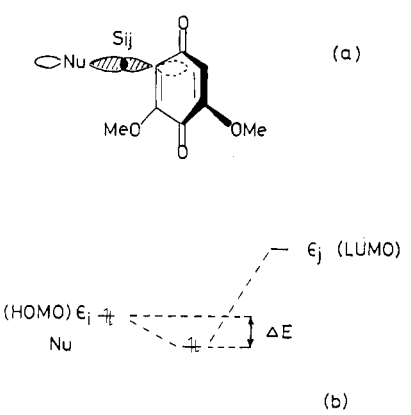

Figure 6. (a) Schematic representation of the orbital overlap between the HOMO of a nucleophile Nu and the LUMO of substituted *p*-benzoquinone. (b) Interaction diagram. ΔE is the stabilization caused by HOMO–LUMO interaction.

entails important steric and electronic consequences for the reaction of **6** with a nucleophile Nu. First, positions 3 and 5 are no longer shielded by the substituents in **6b** and, therefore, **6b** is more prone to nucleophilic attack.

Secondly, bonding HOMO–LUMO interactions between **6** and Nu are stronger in conformation **6b** than in **6a**. This can easily be confirmed with the aid of PMO theory.[15] Upon collision of the two reaction partners, the HOMO of Nu, in most cases an electron lone pair orbital, overlaps with the LUMO of **1**, which is a relatively low lying π–MO. HOMO–LUMO mixing leads to stabilizing two-electron interactions in the collision complex. According to PMO theory, the interaction energy is given by

$$\Delta E = 2(H_{ij} - \epsilon_i S_{ij})/(\epsilon_i - \epsilon_j)$$

where S_{ij} is the overlap integral, H_{ij} the corresponding element of the H-matrix, and ϵ_i and ϵ_j the orbital energies of the HOMO ϕ_i and the LUMO ϕ_j (compare with Figure 6). ΔE increases with increasing HOMO–LUMO overlap and a decreasing difference $\epsilon_i - \epsilon_j$. The larger ΔE is, the more easily bond formation between **1** and Nu will occur.

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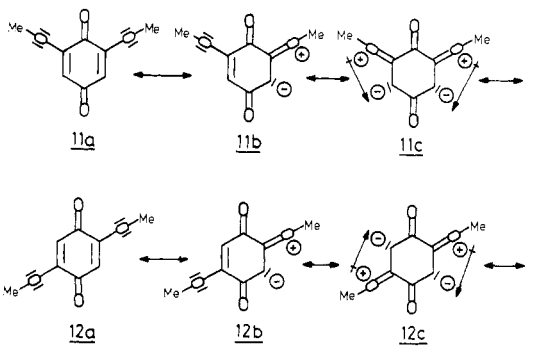

Figure 7. Resonance structures of substituted *p*-benzoquinones **6** and **7**.

Rotation of a methoxy group in **6** and **7** leads to a decrease of the LUMO energy ϵ_j from −1.34 (**6a**) to −1.57 eV (**6b**) and from −1.41 (**7a**) to −1.44 eV (**7b**), i.e., rotation increases HOMO–LUMO interactions. This effect is larger for **6** than for **7**.

As for HOMO–LUMO overlap, methoxy group rotation causes an increase of S_{ij} in the case of **6** but a decrease in the case of **7**. This is revealed by the calculated LUMO coefficients c_{ja} shown in Figure 5. S_{ij} increases with the absolute value of c_{ja}, where the subscript a denotes the position of nucleophilic attack (a = 3 or 5 for **6**; a = 3 or 6 for **7**). We conclude that steric deshielding of ring positions prone to nucleophilic attack leads to enhanced HOMO–LUMO interactions in the case of **6** but only moderate HOMO–LUMO interactions, if any, in the case of **7** (the latter as the result of two opposing effects, namely, a slight increase of $(\epsilon_i - \epsilon_j)^{-1}$ and a reduction of S_{ij}). Furthermore, the calculated LUMO coefficients shown in Figure 5 suggest that overlap is always stronger for **6** than for **7**, i.e., Nu will more easily react with the former quinone.

One can rephrase these results by describing the electronic properties of **6** and **7** with resonance structures **11** and **12** (Figure 7). If **11b**, **11c**, **12b**, and **12c** make a sizable contribution to the electronic features of **6** and **7**, nucleophilic attack at C3 and C5 (C6) will be impeded! For example, a dominance of **11c** and **12c** would lead to repulsive rather than attractive interactions with Nu. Certainly, the importance of resonance structure **c** should be moderate, but it must be higher for **12** than for **11**. In the former case, favorable dipole–dipole attraction (as indicated in Figure 7) will increase the contribution of **c** to the electronic structure of **7** while it should be just the opposite in the case of **6**. Again, we can conclude that **6** reacts more easily with Nu than **7**, which is in line with the observed allergenic properties of these compounds.

Using the same line of arguments and the MNDO results for **10** (n = 1) also shown in Figures 2 and 5, we predict that nucleophilic attack predominantly occurs at the α-position of the methyl rather than the methoxy group. A methyl group is in general only a weak π-donor that obviously becomes even less effective in **10**. In other words, the contribution of a resonance structure similar to **11c** to the electronic structure of **10** is vanishingly small. Also, a resonance structure such as **12c** is hardly of any importance in the case of **8**. Therefore, **8** in contrast to **7** should easily be attacked by a nucleophile. This prediction is in accordance with the sensitizing potency of thymoquinone and related compounds. The stereoplot of the superimposed X-ray geometries (Figure 4) of **2**, **3**, **4**, and **5** exhibits the planar electrophilic quinonoid parts of the molecules. They may fit into a cavity of a monofunctional binding site. The hydrophobic parts of the allergens,

however, require more space, which may be provided by the hypervariable region of light and heavy chains of the receptor protein. This region may also be occupied by long-chained allergens such as **10** (n = 10–15).

Although our results provide a basis for the rationalization of the differing allergenic properties of **6–10**, a caveat is necessary. Compound **10** with n = 1 is known to be a weak allergen while the higher homologoues with n = 2–15 are clearly stronger sensitizers. Experimental sensitization of quinones **10** has revealed that the maximum of allergenicity is reached for n = 11.[16] This is in good agreement with the dependence of allergenic behavior on the length of the side chain in urushiol derivatives known to be active in poison ivy and poison oak.[17,18] Both observations are contrary to the common view that an increase of the side chain leads to increased steric shielding of the p-benzoquinone ring. Also, there is no reason to believe that an increase of the side chain will influence the electronic properties of the ring. Therefore, the experimental evidence suggests the influence of a third effect, the hydrophobic character of the side chain, which increases with the length of the latter and facilitates attractive interactions with the hydrophobic parts of the receptor protein. Furthermore, the binding of sensitizers with long side chains to surface markers of Langerhans cells and/or macrophages may be caused by hydrophobic and other noncovalent interactions between the allergen and side chains of amino acids of the receptor. In addition, the absorption of long-chained quinonoid allergens in the epidermis, especially in the lipid layers of cell membranes, may increase the sensitizing potency of these compounds. This has to be taken into account when trying to rationalize the allergenic character of type **10** structures. Relevant examples for the fact that a longer side chain entails an enhanced allergenic effect are the naturally occurring sensitizers from *Phagnalon saxatile* (2-(dimethylallyl)-1,4-benzoquinone)[19] and *Phacelia* species (2-geranyl-1,4-benzoquinone).[20] The minimum application for allergic reaction was 0.007 μmol for the geranyl-substituted compound and 0.005 μmol for 3-n-pentadecylcatechol,[21] which is known as one of the most potent sensitizers found in the plant kingdom (poison ivy). Compared to these compounds, 2-(dimethylallyl)-1,4-benzoquinone is a relatively weak sensitizer. Its minimum application for allergic reaction is 53 μmol.

Completely substituted quinonoid compounds, e.g., vitamin K_1 (phylloquinone) or anthraquinone, occasionally will show a weak allergenic potency as observed in humans.[22] Also in this case noncovalent interactions between receptor proteins in the epidermis and the quinones may be responsible. The results of the structure determination of the immunoglobulin NEW–vitamin K_1–OH complex support this assumption.[23,24] Saturated (nonelectrophilic) analogues of poison ivy allergens with different side-chain lengths have been tested for their sensitizing properties.[25] Only cyclohexanediols with n-decyl and n-pentadecyl side chains showed moderate sensitizing potency. These results are in line with observations made by Dawson[17] and others.[16]

Conclusion

Experimental observations suggest that the sensitizing capacity of a contact allergen is caused by a nucleophilic attack on the entity O=C—C=CHR. Synthetic p-quinones as well as still-undetected naturally occurring p-quinones must be considered as more or less potent contact allergens when at least one position of the quinone ring is unsubstituted. Increase or decrease of the allergenic potency depends on the number and location of (1) electron-donating substituents and (2) potential sites for nucleophilic attack of SH and NH_2 groups at the quinonoid ring system.

Conformational processes such as the rotation of methoxy groups in **6** (**6a** → **6b**) facilitate nucleophilic attack by steric deshielding of positions 3 and 5 and by increasing HOMO–LUMO interactions between Nu and quinone. The latter is not true for **7**, which should react less easily with a nucleophile than **6**. Compound **7** is known to be nonallergenic while **6** is a moderate contact allergen.

We predict that p-benzoquinones with substituents such as OH,[26] OR, F, Cl, R, etc., at positions 2 and 5 (or 3 and 6) will show no sensitizing potency, while 2,6-disubstituted p-benzoquinones should exhibit allergenic activity. This has to be examined by sensitization and eliciting tests.

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Registry No. **2**, 15121-94-5; **3**, 3755-64-4; **6**, 530-55-2; **7**, 3117-03-1; **8**, 490-91-5; **10** (n = 1), 611-68-7; **10** (n = 12), 4075-01-8.

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(26) Quinonoid compounds bearing hydroxy groups, e.g., lawson (2-hydroxy-1,4-naphthoquinone) and juglone (5-hydroxy-1,4-naphthoquinone) as well as plumbagin (2-methyl-5-hydroxy-1,4-naphthoquinone),[22] possess irritating rather than sensitizing properties. Hydrogen bonding involving the OH substituent may change the properties and the activity of these compounds. Hausen, B. M., unpublished results.