

A Discriminant Model for Allergic Contact Dermatitis in Anthraquinone Disperse Dyes

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Abstract

Some anthraquinone (AQ) disperse dyes have expressed allergic contact dermatitis (ACD) in some consumer uses while others of the same class have not. One of the puzzling aspects of these haptens is the absence of any obvious protein reactive center. The parent compound is nonallergenic and the Mulliken charges on the carbonyl group of 9 AQ allergens and 10 AQ nonallergens show no significant differences. Moreover, using methylamine as a model for the lysine endgroup, the AM1 computed reaction with AQ is weakly endothermic while that of the known allergen, formaldehyde, is strongly exothermic. As all of the allergenic dyes are red or blue and devoid of any obvious reaction center, these observations tend to suggest an excited state as the reactive species. Accordingly, classical and quantum chemical descriptors were explored together in a two-value regression study with 9 actives, ACD (Class = 1), and 10 inactives, NON (Class = 0).

As in earlier discriminant studies of haptens and nonhaptens, logP was not a factor, nor were the hydrogen-bonding potentials of donor and acceptor groups. However, the summation of the Hammett sigma constants for the AQ ring substituents combined with the computational energy gap between HOMO and LUMO of the radical-anion gave an excellent separation of the two classes. Only one major outlier was observed. The coefficients of the regression equation strongly suggest an electron transfer reaction followed by a nucleophilic radical attack on the cell surface proteins of the epidermal Langerhans cells to mark the beginning of the ACD immune response.

Key words: discriminant analysis, allergic contact dermatitis, anthraquinone dyes, quantum chemical descriptors, immune system, hapten-protein reactivity

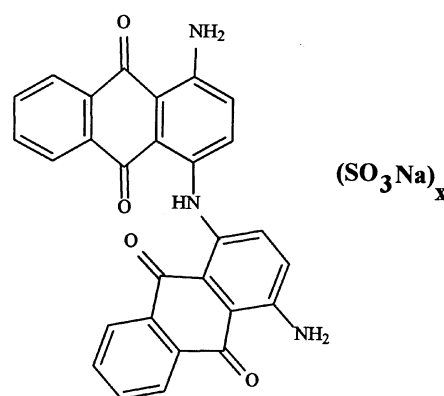
Abbreviations

AQ, anthraquinone; ACD, allergic contact dermatitis; NON, nonallergenic; HOMO, highest occupied molecular orbital; LUMO, lowest unoccupied molecular orbital; DELTA, difference between HOMO and LUMO energies; MHC, major histocompatibility complex.

1 Introduction

Most textile fabrics and other consumer products such as eyeglass frames that come into contact with the skin are colored: they contain dyes. On occasion, these dyes are the cause of allergic

contact dermatitis (ACD). In review articles concerning textile dye contact allergens [1–3], 49 dyes are identified as allergens with twenty (41%) belonging to the azo dye class and eleven (22%) to the anthraquinone class. Anthraquinone textile dyes reported as causing ACD are Disperse Blue 1, 3, 7, 26, 35 and 153, Disperse Red 11 and 15, and Acid Black 48. Other reported anthraquinone dye contact allergens are Disperse Blue 27 [4] and 134 [5]. These dyes were transferred from eyeglass frames and a felt tip marker pen, respectively. The structures of the anthraquinone dyes are provided in Table 1.



Acid Black 48

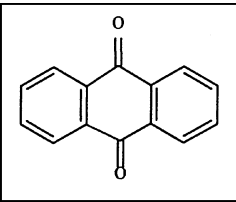
Anthraquinone dyes (AQ dyes) are one of the two most important classes of dyes in use today, the other chemical class being the azo dyes. AQ dyes are those dyes in which the chromophore is the carbonyl group conjugated with two aromatic rings on which amino- and hydroxyl-groups and their substituted forms are frequently present and act as auxochromes*. While the parent AQ is yellow, these modified dyes tend to produce red, violet, blue and green hues. Of the AQ dyes in current use, 83% belong to the disperse application class**, a class comprised of low MW, non-ionic, partially water-soluble dyes that are applied from a micronized dispersion to color synthetic fibers, primarily polyester

(*) A chromophore is the substituent on a dye molecule that creates hue (color) and an auxochrome functions to intensify and/or brighten the hue, to provide solubility in the dyebath, and/or to provide a chemical group that forms an associative bond with the fiber polymers.

(**) Other application classes containing AQ dyes are azoic, acid, basic (cationic), direct (substantive), mordant, reactive, solvent, sulfur and vat. There are no known contact allergens in the azoic, mordant, or sulfur classes.

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Table 1. Dye allergens and nonallergens.

AQ = Anthraquinone 	
<i>Allergens</i>	
Disperse Blue 1	1,4,5,8-Tetraamino AQ
Disperse Blue 3	1-Methylamino-4-(2-hydroxyethylamino) AQ
Disperse Blue 7	1,4-Di(2-hydroxyethylamino)-5,8-dihydroxy AQ
Disperse Blue 26	1,5-Di(methylamino)-4,8-dihydroxy AQ
Disperse Blue 27	1-(4-[2-hydroxyethyl]anilino)-4,5-hydroxy-8-nitro AQ
Disperse Blue 35	2,5-Di(methylamino)-1,8-dihydroxy AQ
Disperse Blue 134 ¹	1,4-Di(isopropylamino) AQ
Disperse Blue 153 ²	70 : 30 mixture of two AQ dyes
Disperse Red 11	1,4-Diamino-2-methoxy AQ
Disperse Red 15	1-Amino-4-hydroxy AQ
Acid Black 48	An imino bis-AQ dye, see structure page 20
<i>Nonallergens</i>	
Disperse Blue 14	1,4-Di(methylamino) AQ
Disperse Blue 56/2 ³	2-Chloro-1,5-diamino-4,8-dihydroxy AQ
Disperse Blue 56/3 ³	3-Chloro-1,5-diamino-4,8-dihydroxy AQ
Disperse Blue 72	1-(4-Methylanilino)-4-hydroxy AQ
Disperse Blue 73	1,5-Diamino-4,8-dihydroxy-6-(4-methoxyphenyl) AQ
Disperse Red 4	1-Amino-2-methoxy-3-hydroxy AQ
Disperse Red 9	1-Methylamino AQ
Disperse Red 22	1-Anilino AQ
Disperse Red 60	1-Amino-2-phenoxy AQ
Disperse Red 86	1-Amino-2-methoxy-4-(4-methylbenzenesulfamido) AQ

¹ Also named Solvent Blue 36.² Not included in model.³ Disperse Blue 56 may also contain Br in place of Cl.

fiber. The disperse class dyes generally exhibit poor wetfastness, that is, they have a high potential to transfer from fabric to wash water or from fabric to skin under moist conditions. Disperse dyes also tend to rub off the surface of fibers to which they were applied, a phenomenon called crocking.

AQ dyes pose a conceptual problem in contact dermatitis in that no protein reactive group is evident in those dyes to cause the immune response of ACD. In all previous studies in our labs [6–10] and by others [11–16], the protein reactive groups are well defined. The mechanism of reaction involves penetration to the viable epidermis and subsequent reaction of the hapten with the cell surface molecules of the Langerhans cells to form the antigen that triggers the immune response [17]. Protein reactivity on the MHC Class II recognition site is limited to electrophiles, strong nucleophiles and some metabolically produced radicals. Neutral AQ dyes are clearly not nucleophiles and while the carbonyl groups are electrophilic, they are not impressive in chemical reactivity. Anthraquinone, the parent molecule, is not known to induce an ACD response, though a single case of sensitization has been reported for the related benzophenone [22]. Part of this study will compare the relative reactivity of anthraquinone, fluorenone and benzophenone with the known allergen, formaldehyde. The photoexcitation of these dyes and the lack of obvious reactivity in the ground state suggests an electronic mechanism proceeding through a reactive intermediate.

To improve our chances of distinguishing between ACD reactive AQ dyes and nonreactive dyes, we have employed quantum chemical descriptors that are electronically based.

Aside from these special descriptors, the technique for discrimination is identical to that in our defining paper on modelling allergic contact dermatitis, namely, two-value regression analysis [6]. We have found this method to have several advantages over the parallel method of linear discriminant analysis.

2 Purpose

Part of this study compares the relative reactivity of anthraquinone and the related fluorenone and benzophenone with the known allergen, formaldehyde. This is done by computing the heat of the model reaction with methylamine and has the purpose of establishing the improbability of lysine attack on the carbonyl group of the AQ dyes. With this established, the photoexcitation of these dyes and the lack of obvious reactivity in the neutral ground state strongly suggests an electronic mechanism proceeding through a reactive intermediate. This indication leads us to thoroughly explore the possibility of electron capture as a significant route to protein reactivity.

3 Methods

3.1 Selection of dyes

Antraquinone dyes thought to induce an ACD response in some individuals were assembled from published case reports of dermatologists and compared with related AQ dyes believed to be benign in consumer use (Table 1). By eliminating redundancies of molecular structure and those with complex or uncertain structures beyond the range of the set, it was possible to define 9 allergens and 10 nonallergens for the purpose of a discriminant analysis. This is a relatively small set as our primary study of ACD response contained 72 compounds (36 ACD, 36 NON) [6]. It is also fuzzy in the statistical sense as the minimum criteria for placing a dye into the ACD category was, in some cases, just one case report. There are, however, supporting clinical studies on many of these dyes [18], as well as recent studies employing the local lymph node assay (LLNA) of Kimber *et al.* [20] and a modification thereof [18]. These studies are mostly corroborative for the strong sensitizers, but less so for the weaker sensitizers where the LLNA procedure is less able to define active from inactive on a scale using an arbitrary point of minimum response. While this does not compare in confidence with the clinical data used in other studies, it is what we have to work with and represents a clear challenge in discriminant analysis.

3.2 Descriptors for discriminant modelling

Two sets of descriptors were explored for discriminating potential. The first set of descriptors is based on simple counts of the hydrogen-bonding potential of each dye, the calculated logP (octanol/water), and the sum of the Hammett sigma constants of the ring substituents. These are traditional QSAR descriptors coding for H-bonding effects, transdermal transport (logP), and for non-specific electronic effects centered on the anthraquinone rings. The acceptor (HBA) and donor (HBD) counts are corrected for intra-molecular H-bonding, which reduces the available sites for external interaction.

The descriptors and definitions are as follows: HBA, count of electron pairs on O and N, less those involved with the AQ carbonyl groups; HBD, count of N-H and O-H donor bonds, less those involved with the AQ carbonyl groups; logP (octanol/water), estimated with the CLOGP program; RNSIG, a summation of the Hammett sigma constants for each of the ring substitution groups on the most electron-donating ring. The powerful effect on color of the 1, 4, 5, 8 groups suggests the use of para constants. The weaker 2, 3, 6, 7 groups are assigned meta constants.

The other set of descriptors is electronically based and follows the concept that no obvious center of protein reactivity exists on the AQ dyes. These descriptors are derived from the AM1 semiempirical modelling of each dye, some of which required many hours for convergence on a Pentium 150 MHZ computer running Hyperchem 4.5. The slow convergence is related to the flexibility of many of the AQ dye substituents, rather than the average molecular size of the dyes. These descriptors address a different type of binding (dipole moment), electrophilic reactivity of the carbonyl group (C=O carbon charge), thermodynamic heat of electron acceptance (δH_r), the energetics of the nucleophilic (HOMO) and electrophilic (LUMO) centers of the dyes as well as their differences (DELTA) for both the ground state dye and the resulting radical-anion.

The descriptors and definitions are as follows: Dipole Moment, derived from AM1 model of dye, Debyes; C=O charge, sigma charge on carbon atom, AM1 model; Hf(neut), heat of formation of ground state dye, AM1, kcal/mol; Hf(ion-rad), heat of formation of radical anion, AM1, kcal/mol; Hrxn, heat of electron acceptance, [Hf(ion-rad) – Hf(neut)], kcal/mol; EHOMO, electronic energy of highest occupied orbital for the neutral AM1 model, electron volts*; EHOMORAD, electronic energy of highest occupied orbital for the AM1 radical-anion, electron volts*; ELUMO, electronic energy of lowest unoccupied orbital for the neutral AM1 model, electron volts*; ELUMORAD, electronic energy of lowest unoccupied orbital for the AM1 radical-anion, electron volts*; DELTA, [ELUMO – EHOMO], electron volts*; DELTARAD, [ELUMORAD – EHOMORAD], electron volts*;

* [Note: 1 electron volt = 23.06 kcal/mol].

Table 2. Dye descriptors used in discriminant models.

Case	Dye Name	Class	Yest	HBA	HBD	logP	RNSIG
1	Disperse Blue 1	1	1.024	4	4	–0.61	–1.32
2	Disperse Blue 26	1	0.980	6	0	1.02	–1.21
3	Disperse Blue 27 ¹	1	0.544	10	1	2.03	–0.77
4	Disperse Blue 134	1	0.921	4	0	3.10	–1.22
5	Disperse Blue 3	1	1.069	6	1	1.15	–1.45
6	Disperse Red 11	1	0.870	6	2	0.62	–1.20
7	Disperse Red 15	1	0.761	5	1	1.38	–1.03
8	Disperse Blue 35	1	0.937	7	1	1.02	–1.21
9	Disperse Blue 7	1	0.965	10	2	0.14	–1.22
10	Disperse Blue 72	0	0.224	5	0	3.88	–0.77
11	Disperse Red 86	0	–0.289	8	1	6.66	–0.73
12	Disperse Blue 73	0	0.360	8	3	1.71	–1.03
13	Disperse Red 60	0	0.069	6	1	3.09	–0.69
14	Disperse Blue 14 ²	0	1.371	4	0	1.59	–1.69
15	Disperse Blue 56/3 ²	0	0.705	6	2	0.83	–1.03
16	Disperse Blue 56/2 ²	0	0.715	6	2	1.10	–1.03
17	Disperse Red 22	0	–0.132	4	0	3.69	–0.40
18	Disperse Red 9	0	0.574	4	0	2.02	–0.84
19	Disperse Red 4	0	0.123	8	2	1.12	–0.42

Table 2. (continued)

Case	Dipole	C = O Chg	Hf(neut)	Hf(ion-rad)	Hrxn	EHOMO
1	0.00	0.336	−4.45	−36.13	−31.68	−7.324
2	0.00	0.339	−88.08	−131.04	−42.96	−8.016
3	8.16	0.324	2.48	−64.21	−66.69	−7.622
4	1.65	0.320	−20.41	−61.00	−40.59	−7.924
5	3.25	0.323	−46.24	−86.21	−39.37	−8.283
6	3.48	0.323	−44.28	−80.06	−35.78	−7.706
7	2.03	0.322	−52.00	−94.22	−42.22	−8.368
8	3.28	0.328	−78.63	−133.91	−55.28	−8.035
9	2.93	0.335	−194.62	−241.45	−46.83	−8.240
10	1.94	0.321	−10.80	−55.90	−45.10	−8.056
11	2.73	0.328	−79.85	−132.08	−52.23	−8.195
12	2.85	0.340	−91.42	−134.24	−42.82	−7.941
13	1.57	0.324	−6.32	−52.30	−45.98	−8.547
14	2.39	0.319	1.60	−36.08	−37.68	−7.676
15	0.88	0.341	−96.92	−140.24	−43.32	−8.157
16	0.80	0.341	−85.28	−129.96	−44.68	−8.184
17	0.99	0.322	34.96	−10.45	−45.41	−8.302
18	2.16	0.324	−4.57	−44.22	−39.65	−8.534
19	3.11	0.329	−81.28	−120.07	−38.79	−8.518

Table 2. (continued)

Case	ELUMO	DELTA	EHOMORAD	ELUMORAD	DELTARAD
1	−0.847	6.477	0.500	3.831	3.331
2	−1.296	6.720	0.000	3.425	3.425
3	−2.333	5.289	−0.820	2.555	3.375
4	−1.129	6.795	0.196	3.508	3.313
5	−1.024	7.259	0.193	3.400	3.207
6	−1.042	6.664	0.389	3.647	3.258
7	−1.351	7.017	0.188	3.525	3.337
8	−1.102	6.933	−0.075	3.279	3.354
9	−1.386	6.854	−0.181	3.205	3.386
10	−1.359	6.697	−0.020	2.823	2.843
11	−1.304	6.891	−1.229	1.825	2.054
12	−1.294	6.647	−0.116	2.556	2.672
13	−1.264	7.283	0.021	2.730	2.709
14	−1.105	6.571	0.330	3.671	3.341
15	−1.345	6.812	0.046	3.291	3.245
16	−1.391	6.793	−0.013	3.248	3.261
17	−1.397	6.905	0.002	2.821	2.819
18	−1.235	7.299	0.341	3.658	3.317
19	−1.186	7.332	0.321	3.532	3.211

¹ Failed to converge. Computations done at gradient = 0.440.² Not included in discriminant equation.

ACD, the active set of AQ dyes has a Class Value = 1; NON, the inactive set of AQ dyes has a Class Value = 0.

All of the descriptors for each AQ dye are collected in Table 2.

3.3 Statistical methods

By preference, we selected the discriminant method of two-value regression analysis as our earlier experience showed several advantages over linear discriminant analysis [6]. In this method, the ACD dyes are coded Class 1 and the NON-ACD dyes are coded Class 0. The regression program in MINITAB 6.1 was used in this study [19].

In Eqs. 1 and 2, the Student T values are in parentheses after each descriptor. These values are the ratio of the coefficient to the standard error of the coefficient, for example, a T value of 2.00

represents a 50% error in the coefficient and is considered of minimal significance. The T values are mathematically equivalent to the 95% CL of the coefficient, but are visually easier to interpret. The number of data points is represented by n, the scatter about the regression line (Y estimate) by s, the explained variance by r^2 , and the overall strength of the equation by F, the Fisher distribution. F values greater than 10 are highly significant. The correlation coefficient reported in many studies is derived from the explained variance and, while more impressive in magnitude, has less significance.

3.4 Quantum mechanical methods

To evaluate the potential protein reactivity of the ketonic group of the AQ dyes, comparative semiempirical studies were made with a simple nucleophile representing the terminal group of lysine. Anthraquinone, fluorenone, benzophenone and the known hapten,

Table 3. Estimated heats of reaction of formaldehyde, benzophenone, fluorenone and anthraquinone by AM1 modelling.

Keto Compound	Hf (keto cpd) ¹	Hf (adduct)	Hr (kcal/mol)
Formaldehyde	−31.53	−53.19	−14.19
Benzophenone	22.52 ²	17.46	2.68
Fluorenone	34.14	27.37	0.70
Anthraquinone	−6.32	−9.75	4.04

¹ The AM1 Hf(methylamine) = −7.47 kcal/mol.² Benzophenone converges to a local minimum with a planar structure (28.65 kcal/mol) unless the starting geometry is orthogonal.

formaldehyde, were included in the study. The model nucleophile is methylamine and the program chosen to develop computational estimates of the heats of formation is AM1, the same program used to study the heats of electron acceptance of the AQ dyes. All compounds are modelled to a minimum energy state and the heats of reaction are calculated simply as the difference in heats of formation of the reactants and the first intermediate, ArC(OH)(NHCH₃). These are gas-phase evaluations, but as all intermediates experience similar solvation forces, the relative order is expected to indicate the relative reactivity of these ketonic compounds with the end-group of lysine.

4 Results

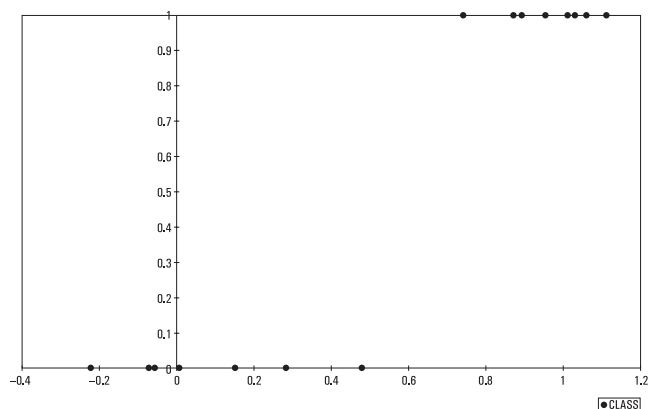
4.1 Protein reactivity of anthraquinone

Modelling the reaction of methylamine with formaldehyde, benzophenone, fluorenone and anthraquinone to evaluate relative heats of reaction is summarized in Table 3. The estimated heat of reaction of methylamine with formaldehyde (−14.9 kcal/mol), a known sensitizer and protein reactive embalming agent, is strongly exothermic, while that of benzophenone (2.68 kcal/mol), fluorenone (0.70 kcal/mol) and anthraquinone (4.04 kcal/mol) are all weakly endothermic. The energetic difference between the formaldehyde and AQ reactions is 18.23 kcal/mol, removing any possibility of protein reactivity at the AQ keto group. These estimated heats of reaction were central to the exploration of the AM1 electronic values, as no protein reactive center was apparent on the AQ dyes.

In addition to this result, the computed positive charge on the carbonyl groups of the 19 AQ dyes showed no discriminating power between ACD and NON, the total range of variance being 0.319–0.341.

4.2 Discriminant modelling of the anthraquinone dyes

A simple correlation matrix revealed that several of the descriptors were lacking in individual discriminating power and were likely to be eliminated in the modelling process. In addition to C=O charge ($r=0.074$), these included both heats of formation, the heat of electron acceptance, HBA, HBD, ELUMO and EHOMORAD, none of which exceeded $r=0.2$. For a large array of descriptors, the most efficient process is to regress all followed by careful backward selection until the model reaches sufficient strength to evaluate outliers.

**Figure 1.** Class versus Y estimate for equation 1.

Only 3 of the 16 descriptors explored were significant in separating the characteristics of the AQ dye allergens from those of the nonallergens. In the statistical process, 3 outliers were detected and deleted, each of which are classed as non-allergen. The remaining 7 nonallergens are cleanly separated from the 9 AQ allergens in a statistically strong relation (Eq. 1). Figure 1 shows the separation of the Y estimate plot against the class value. Note the very narrow distribution of the active class relative to that of the nonallergenic dyes. The residual values show reasonable central tendency for a small set but the distribution is skewed.

$$\begin{aligned} \text{CLASS} &= 0.878 \cdot \text{DELTARAD}(4.46) - 0.419 \cdot \text{EHOMORAD}(2.72) \\ &\quad - 0.928 \cdot \text{RNGSIG}(4.60) - 3.07 \\ n &= 16 \quad s = 0.219 \quad r^2 = 0.854 \quad F = 23.42 \quad [1] \end{aligned}$$

Although the separation is clean and the statistics are strong, the distribution of the Y estimate *versus* Class plot and the skewed residuals is reason for reflection. The inclusion of EHOMORAD is also questionable as it showed no discriminating power against Class in the correlation matrix ($r=0.093$). Removal of EHOMORAD from the model leads to a weaker but more realistic correlation revealing the same 3 outliers (Eq. 2). In addition, the separation is no longer artificially perfect, the two classes are more equally distributed (Figure 2) and the residuals closely approach a normal distribution.

$$\begin{aligned} \text{CLASS} &= 0.603 \cdot \text{DELTARAD}(2.92) - 0.921 \cdot \text{RNGSIG}(3.74) \\ &\quad - 2.20 \\ n &= 16 \quad s = 0.267 \quad r^2 = 0.764 \quad F = 21.06 \quad [2] \end{aligned}$$

By Eq. 2, the 3 outliers: Disperse Blue 56/2, Disperse Blue 56/3, and Disperse Blue 14 have predicted Y estimates of 0.72, 0.71, and 1.36 respectively. All predict ACD activity. As Disperse Blue 56/2 and 56/3 are virtually identical in structure (Table 1), they represent a group outlier. The retention of one or both in the equation would represent a misclassification of only 5–10%, which is quite acceptable in this type of discrimination. Disperse Blue 14 is a more serious problem as the discrepancy from the classification mean ($1.36 - 0.50 = 0.86$) is so large that it challenges the basis of the model.

Replacing the Hammett RNGSIG values with equivalent sigma(+) constants leads to a similar but somewhat weaker correlation

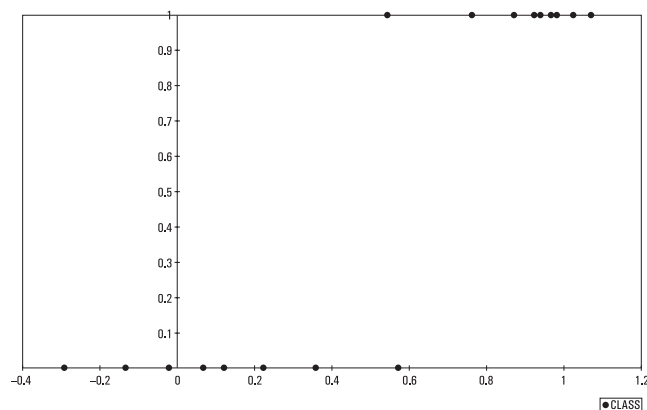


Figure 2. Class versus Y estimate for equation 2.

($r^2 = 0.735$, $F = 17.99$). This important test leads to a firm conclusion regarding the mechanism of formation of the radical-ion as noted in the discussion.

5 Discussion

All of the selected AQ disperse dyes reported to cause ACD are blue or red, which in itself, suggests some form of photoactivation or related electronic process. In order to develop an unbiased model, all of the nonallergenic AQ dyes were also selected from the blue and red class. As a statistical model of this size ($n = 16$ – 19) can only support 2–3 of the most important descriptors, it seemed logical to include variables related to electronic properties. A second point of interest was the elimination of the carbonyl group as an electrophilic center for the hard nucleophiles lysine and activated serine. This was done by modelling the reactivity of AQ, fluorenone and benzophenone in comparison with formaldehyde, a known strong allergen. As Table 3 shows, the carbonyl reactivities of AQ, fluorenone and benzophenone with primary amine (lysine analog) are strongly endothermic with respect to formaldehyde. This is supported by a literature search showing no evidence that these compounds are skin sensitizers with the exception of a single report on benzophenone [22]. In addition, Table 2 shows that the computed ground state charge on the carbonyl carbon of allergenic and nonallergenic dyes is virtually identical and therefore, not a discriminant factor.

As in earlier modelling studies [6–8], logP did not prove to be a factor. However, in contrast to these same studies, neither form of hydrogen-bonding, HBA or HBD, was significant. Despite a very large range in values (0 – 8.16 Debyes), the dipole moment was also not a factor.

The descriptors of the best two-value regression equation are both electronic in nature and strongly suggest electron acceptance to a radical-anion and subsequent nucleophilic attack. The two strongest factors are DELTARAD of the radical-anion and the sum of the ring substituent sigmas, RNGSIG. As DELTARAD has a positive coefficient, AQ dyes expressing ACD tend to have larger values than the nonallergenic dyes. The range of values for the ACD dyes is 3.207–3.425 while that of the NON dyes is 2.054–3.341. The significance of this unusual descriptor implies the intermediacy of the AQ radical-anion in the hapten-protein

reaction. The coefficient of RNGSIG clearly suggests that some form of nucleophilic reaction is supported by electron donating groups. The source of the electron transfer for this mechanism is unknown, as is the target of the nucleophilic attack. However, the structure of the AQ dyes is not unlike some of the known aromatic electron acceptors in normal biochemistry, such as flavin mononucleotide and ubiquinone. Whether protein reactivity proceeds by 2-electron or 1-electron bonding is also unknown. If the process is radical in nature (1-electron), then the possibility of photoactivation to a reactive singlet or triplet needs to be explored and this work is in progress. The somewhat weaker correlation of sigma(+) versus Hammett's sigma tends to eliminate an alternate route (electron loss) to a radical cation. As most of the ring substituents have very strong sigma(+) values, we would expect this mechanism to be strongly revealed. We are indebted to one of the referees for suggesting this experiment.

Lastly, it is necessary to address the statistical outliers that reduce the original set from $n = 19$ to $n = 16$. All three are rated nonallergenic and two of them are virtually identical isomers, Disperse Blue 56/2 and 56/3, which differ only in the position of the halogen on the ring. Neither of these points are serious outliers ($Y \text{ est.} = 0.71, 0.72$) and the model would have remained valid, through weaker ($r^2 = 0.662$, $F = 9.14$) without deletion. Disperse Blue 14, however, is grossly overpredicted ($Y \text{ est.} = 1.37$) with properties in concordance with the class of dye allergens. There is no immediate explanation for this discrepancy, but it will prompt an investigation to determine if this dye is correctly classed as a nonallergen. With these deletions, all 16 of the remaining dyes are separated by the model with only minor overlap. In larger studies [6–8] some overlap in the Y estimate is generally observed. If a larger set of dyes were available, the overlap would probably bring Blue 56/2,3 into the statistical fold rather than identifying these points as outliers. While a larger study might accommodate these two, Blue 14 would be a serious outlier in any set.

In summary, we have shown that electrophilic reactivity of the AQ disperse dyes is most unlikely and that some form of activation by electron transfer or photopromotion is responsible for protein reactivity and sensitization. The effect of the AQ ring substituents strongly suggests a nucleophilic attack by a carbonyl oxygen radical-anion. We speculate that this would be *via* a nucleophilic radical attack on tyrosine or tryptophan as the O–S bond is too weak to permit an effective nucleophilic reaction with a cystine group.

6 References

- [1] Hatch, K. L., *Textile Res. J.* 54, 664–682 (1984).
- [2] Hatch, K. L. and Maibach, H. I., *J. Am. Acad. Dermatol.* 32(4), 631–639 (1995).
- [3] Hatch, K. L., in: Surber, C., Elsner, P. and Burcher, A. J. (Eds.), *Exogenous Dermatology: Advances in Skin Related Allergology, Bioengineering, Pharmacology and Toxicology*, Karger, Basel 1995, pp. 8–15.
- [4] Dooms-Goossens, A., Bonamie, A., Parys, M. and Dooms, M., *Contact Dermatitis* 7, 214–215 (1981).
- [5] Miller, M. M., Goldberg, H. S. and Wilkerson, W. G., *Arch. Dermatol.* 114, 1793–1794 (1978).
- [6] Magee, P. S., Hostynek, J. J. and Maibach, H. I., *Quant. Struct.-Act. Relat.* 13, 22–33 (1994).

- [7] Magee, P. S., Hostynek, J. J. and Maibach, H. I., in: Salem, H. (Ed.), *Animal Test Alternatives*, Marcel Dekker, New York 1995, pp. 159–166.
- [8] Magee, P. S., Hostynek, J. J. and Maibach, H. I., in: Rougier, A., Goldberg, A. M. and Maibach, H. I. (Eds.), *In Vitro Skin Toxicology: Irritation, Phototoxicity, Sensitization*, Mary Ann Liebert, New York 1994, pp. 281–291.
- [9] Magee, P. S., in: Hansch, C. and Fujita, T. (Eds.), *Classical and Three-Dimensional QSAR in Agrochemistry*, ACS Symposium Series 606, American Chemical Society, Washington, DC 1995, pp. 120–129.
- [10] Hostynek, J. J., Lauerma, A. I., Magee, P. S., Bloom, E. and Maibach, H. I., *Arch. Dermatol. Res.* 287, 567–571 (1995).
- [11] Dupuis, G. and Benezra, C., *Allergic Contact Dermatitis to Simple Chemicals*, Marcel Dekker, New York and Basel 1982.
- [12] Roberts, D. W. and Williams, D. L., *J. Theor. Biology* 99, 807–825 (1982).
- [13] Barratt, M. D., Basketter, D. A. and Roberts, D. W., *Toxicol. in Vitro* 8, 823–826 (1994).
- [14] Barratt, M. D., Basketter, D. A., Chamberlain, M., Admans, G. D. and Langowski, J. J., *Toxicol. in Vitro* 8, 1053–1060 (1994).
- [15] Basketter, D., Doms-Goossens, A., Karlberg, A.-T. and Lepoittevin, J.-P., *Contact Dermatitis* 32, 65–73 (1995).
- [16] Payne, M. P. and Walsh, P. T., *J. Chem. Info. Computer Sci.* 34, 154–161 (1994).
- [17] Schuler, G., *Epidermal Langerhans Cell*, CRC Press, Boca Raton 1991, pp. 297–298.
- [18] Ikarashi, Y., Tsuchiya, T. and Nakamura, A., *J. Appl. Toxicol.* 16, 349–354 (1996).
- [19] Ryan, B. F., Joiner, B. L. and Ryan, Jr., T. A., *Minitab Handbook*. Second Edition, Duxbury Press, Boston 1985.
- [20] Kimber, I., Dearman, R. J., Scholes, E. W. and Basketter, D. A., *Toxicol.* 93, 13–31 (1994).
- [21] Sailstad, D. M., Tepper, J. S., Doerfler, D. L., Qasim, M. and Selgrade, M. J. K., *Fund. Appl. Toxicol.* 23, 569–577 (1994).
- [22] Ramsay, D. L., Cohen, H. J. and Baer, R. L., *Arch. Dermatol.* 105, 906–908 (1972).