

Structure-activity relationships in the murine local lymph node assay for skin sensitization: α,β -diketones

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The biological activity of skin-sensitizing chemicals is related to their ability to react, either directly or after metabolic activation, with appropriate skin proteins. For direct acting electrophilic compounds, this ability can be modelled, using the RAI (relative alkylation index) approach, by a combination of electrophilicity and hydrophobicity parameters. Several structure-activity relationships based on this approach have been reported, but most of them cover guinea pig sensitization test data on what chemists would classify as relatively soft electrophilic chemicals. In the present work, an electrophilicity parameter based on Taft substituent constants is derived for hard electrophiles having a reactive carbonyl group, and is used to calculate RAI values for the analysis of sensitization test data obtained in the murine local lymph node assay (LLNA) for a series of α,β -diketones. The sensitization potential of these reactive hard electrophilic carbonyl compounds in the LLNA shows a good correlation with the RAI. Overall, the findings reaffirm our view that physical organic chemistry is the key to understanding why some chemicals sensitize more strongly than others, while some do not sensitize at all, and provide further evidence of the value of the LLNA for SAR studies.

Key words: structure-activity relationships; contact allergy; murine local lymph node assay; mouse sensitization; α,β -diketones; relative alkylation index; chemical basis. © Munksgaard, 1999.

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The capacity of a chemical compound to behave as a contact allergen depends, inter alia, on its partitioning properties and its ability to react, either directly or after metabolic activation, with skin proteins. Such reactions, often referred to as protein haptenation, usually involve the sensitizer or its metabolite acting as an electrophile and the protein acting as a nucleophile (1). Based on this concept, it has proved possible to establish structure-activity relationships correlating skin sensitization potential with physicochemical parameters for a variety of classes of chemicals, in a variety of sensitization assays (2).

The recently-developed murine local lymph node assay (LLNA) has several advantages over longer established sensitization test assays (3, 4). Successful application of the LLNA to develop mechanism-based structure-activity relationships can be valuable, not just because of the relationships themselves, but for the demonstration they

can provide of the scope and applicability of the LLNA for risk assessment purposes.

Here we report LLNA results for a set of α,β -diketones. Although there are several well-known sensitizers belonging to this chemical class (1), structure-sensitization relationships for compounds of this type have not been reported until now.

Haptenation Reactions of Carbonyl Compounds

Activation by neighbouring electron-withdrawing groups appears to be necessary to make carbonyl groups in ketones reactive enough to sensitize (1). Since the carbonyl group is a hard electrophilic centre, the haptenation reactions leading to sensitization are unlikely to involve cysteine groups, which are soft nucleophiles, but can occur via reaction with primary amine groups associated with lysine units in proteins, forming Schiff's bases (1).

Guanidino groups of arginine units can also react in an analogous manner, particularly when 2 carbonyl groups are bonded directly together (α,β -dicarbonyl compounds), as in glyoxal (5) and diacetyl (6), both of which are known sensitizers. Other known α,β -dicarbonyl sensitizers can arise from oxidation or dehydration of steroidal hydroxyketones (7). The reaction with guanidino groups in such cases can give rise to heterocyclic structures, as discussed by Bundgaard (7). These sensitizers, and their proposed reactions with skin proteins, are shown in Fig. 1.

Structure-Reactivity Modelling

Irrespective of the exact nature of the haptenation reaction, the electrophilic reactivity of the most electron-deficient carbonyl group will determine how much of the compound available for reaction becomes chemically bound to skin protein. The amount of compound available depends on the dose given and on the compound's partitioning properties.

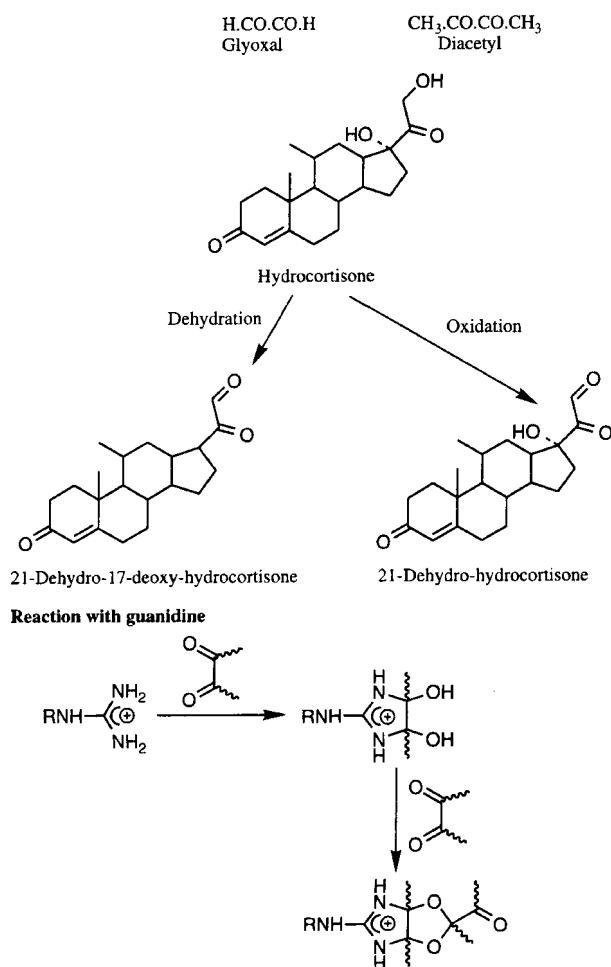
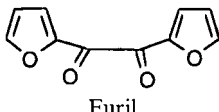
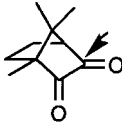


Fig. 1. α,β -dicarbonyl sensitizers.

Table 1. Compounds tested and their physicochemical properties

Compound	RP	log P	M.pt.	LNNA result
 Furil	0.50	1.42	164°C	5% 1.2 10% 1.7 25% 2.2
 Camphorquinone	1.62*	0.69	200°C	5% 2.8 10% 3.0 25% 1.7
MeCOCOMe butane-2,3-dione	1.81	-1.22	<20°C	5% 1.4 10% 2.8 25% 5.2
PhCOCOMe 1-Phenylpropane-1,2-dione	2.56**	1.02***	84°C	5% 12.8 10% 17.7 25% 20.1

* RP based on σ^* values for MeCO and i-Pr as surrogates for alicyclic keto and alkyl groups. Reaction assumed to take place at site arrowed. This RP value probably somewhat overestimates the relative reactivity, since MeCO should be more electronegative than the carbonyl groups in camphorquinone.

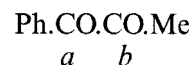
** See text for details of RP calculation.

*** From ClogP programme. Other log P values calculated manually by the Leo & Hansch (9) method.

The electrophilic reactivity of a carbonyl group depends on the degree to which the carbonyl carbon atom bears a partial positive charge. Electron donating groups attached to the carbonyl group deactivate it (dialkyl ketones are less reactive than aldehydes because alkyl groups are electron donating) and electron attracting groups activate it. A simple way to quantify these electronic effects is by means of Taft σ^* substituent constants.

The Taft σ^* constant for a substituent X is a measure of the inductive effect of X. The σ^* values used here are taken from the extensive compilation by Perrin et al. (8).

We can now define a reactivity parameter RP for carbonyl compounds as the sum of the σ^* constants for the 2 groups attached to the most electron deficient carbonyl group. This is illustrated by the case of 1-phenylpropane-1,2-dione:



Relevant σ^* values are: Ph, 0.75; PhCO, 2.20; Me, 0.00; MeCO, 1.81.

For carbonyl group a, RP is given by $\sigma^*(\text{Ph}) + \sigma^*(\text{COMe}) = 0.75 + 1.81 = 2.56$.

For carbonyl group b, RP is given by $\sigma^*(\text{PhCO}) + \sigma^*(\text{Me}) = 2.20 + 0.00 = 2.20$.

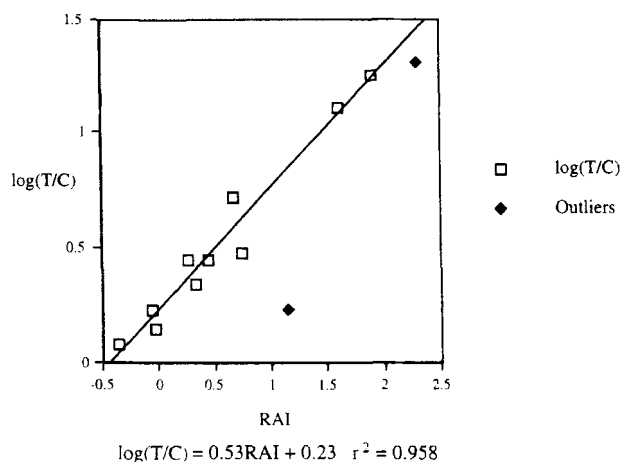


Fig. 2. QSAR/dose response relationship for diketones.

RP for carbonyl group *a* is larger than RP for carbonyl group *b*, indicating that carbonyl group *a* is more electron deficient than carbonyl group *b*. Therefore the RP value of 2.56, corresponding to carbonyl group *a*, is used for 1-phenylpropane-1,2-dione.

Application to a Test Data Set

A set of 4 α,β -diketones, which had been tested in the course of a product development research programme, provided a set of LLNA data suitable for structure-activity analysis. Table 1 shows their chemical structures, their RP values (from σ^* values tabulated in ref (8)), their log *P* values and the LLNA response data, the latter expressed as the stimulation index *T/c* (the ratio of proliferation in a test group compared to that in concurrent vehicle treated controls). Whilst a stimulation index of 3 is taken as the cut-off for point for the identification of a positive result in the LLNA, this is in respect of formal regulatory classification (3). For scientific purposes, it is reasonable to make use of the whole dose-response curve, as has been done previously in QSAR studies based on LLNA data (4). Melting points (from suppliers' catalogues) are also shown. On the basis of their log *P* values, all of the compounds would be expected to penetrate skin readily.

Since there are only 4 compounds but 12 LLNA results, this data set is most appropriately modelled by an RAI-based combined QSAR/dose-response relationship. Fig. 2 shows a plot of log (*T/C*) against RAI, the RAI values being calculated as:

$$RAI = \log D + RP + 0.5 \log P$$

where *D* is the % concentration applied divided by the molecular weight.

Fig. 2 shows a clear relationship between the LLNA response, as quantified by log (*T/C*), and the RAI calculated on the above basis. 2 of the 12 data points are omitted from the regression analysis and are treated as outliers. 1 of these corresponds to camphorquinone tested at 25%. Camphorquinone, which on the basis of the test results would be classified as, at most, marginally positive, does not show a typical dose-response relationship, the response at the highest concentration (25%) being lower than the responses at the other concentrations. In view of the overall low level of these responses, an overload effect seems very unlikely. We note, however, that camphorquinone has a very high melting point (200°C). Possibly at the 25% concentration it precipitates out of solution during passage through the stratum corneum.

The other outlier corresponds to 1-Ph propan-1,2-dione, the strongest sensitizer of the 4 compounds studied, tested at 25%. The *T/C* figure, although higher than that obtained at 10%, is somewhat less than would be expected based on extrapolation from the 5% and 10% scores and, attributing this to the onset of overload, we decided to omit it from the regression.

In conclusion, the findings presented here demonstrate that, as found for other types of electrophilic skin sensitizers, the sensitization potential of reactive carbonyl compounds is well modelled by the RAI based on reactivity and hydrophobicity parameters. Overall, the findings reaffirm our view that physical organic chemistry is the key to understanding why some chemicals sensitize more strongly than others, while some do not sensitize at all, and provide further evidence of the value of the LLNA for SAR studies.

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