



STRUCTURE-ACTIVITY RELATIONSHIP AS A TOOL IN CONTACT ALLERGY RISK ASSESSMENT

A.-T. KARLBERG*, J. L. G. NILSSON† and J. E. WAHLBERG*

*Department of Occupational Dermatology, National Institute of Occupational Health, S-171 84 Solna, Sweden and Karolinska Hospital, S-104 01 Stockholm and †Department of Organic Pharmaceutical Chemistry, Box 574, Uppsala University, Uppsala, Sweden

Summary—About 3000 substances are known to cause contact allergy (type IV allergy). The possibility of predicting the sensitizing capacity of a substance by examining its chemical structure is discussed on the basis of the authors' own experience and of literature data. Since other factors also influence the allergenic activity of a compound, indications based on chemical considerations must be confirmed experimentally in animals because no *in vitro* methods yet exist.

Introduction

Is it possible to predict the sensitizing capacity of a substance by examining its chemical structure? This question will be discussed on the basis of experience from our own research and of literature data.

Some 3000 compounds are known to cause contact allergy (type IV allergy) (de Groot, 1986). Most of these are chemically defined substances, whereas for type I allergy only about 30 chemically well defined substances are known. Every year new allergens are detected in clinical practice and in research work.

In the initial steps of the development of contact allergy, compounds with low molecular weight (haptens) penetrate the skin and bind covalently to skin proteins to form complete antigens. The functional hapten also may be formed from a prohaptens by metabolism.

Chemical properties of haptens

To cause contact allergy the substance must be able to penetrate into the skin. Lipid-soluble molecules with a certain degree of water solubility are the most active. The molecular weight must be low—as a rule not more than 500. Moreover, the compound must have chemical properties that favour binding to skin proteins.

Nucleophiles and electrophiles. The basic rule is that a compound able to cause contact allergy must have electrophilic properties (Dupuis and Benezra, 1982). The skin can be described chemically as a nucleophilic (rich in electrons) environment. It contains water and

macromolecules with electron-rich positions such as thiol (-SH), amino (-NH₂) and alcohol (-OH) groups (e.g. in cysteine, lysine, histidine and tyrosine). These groups are nucleophiles and can react with electron-deficient positions (electrophilic) in other molecules. Therefore, electrophilic properties are essential for the ability of a compound to bind to proteins. However, the protein bond can also be formed through a radical mechanism (Fig. 1).

When a hapten containing electrophilic positions penetrates the skin and binds to amino or thiol groups in a protein, the protein will be modified (Fig. 1). It will no longer be recognized as 'self', but will act as an antigen.

Prohaptens. Enzymatic processes in the skin or chemical transformation outside the skin can change the structure and make some substances allergenic that are not originally so. Such substances are designated prohaptens (Dupuis and Benezra, 1982). The conversion from a prohaptens to a hapten might take place by oxidation involving enzymes in the skin (among others cytochrome P-450). The oxidation can be due also to oxygen in air, often in combination with light or heat (Fig. 2). In our research work we have studied how mono- and diterpenes, which in themselves are not allergenic, are transformed to active haptens by air oxidation (Gäfvert *et al.*, 1992; Karlberg *et al.*, 1985, 1988, 1991, 1992 and 1994). Some of the haptens created are able to bind to proteins by way of a radical mechanism.

Three allergenic metals. The metals nickel, cobalt and chromium are among the most common causes of contact allergy, whereas other metals, such as iron, zinc and silver, are almost never implicated, although skin exposure occurs. There is no complete explanation for this, but it is believed that nickel, cobalt and chromium are able to form co-ordination complexes with certain ligands in the proteins. The complexes might cause changes in the conformation of

*Department of Occupational Dermatology, National Institute of Occupational Health, S-171 84 Solna, Sweden and Karolinska Hospital, S-104 01 Stockholm and

†Department of Organic Pharmaceutical Chemistry, Box 574, Uppsala University, Uppsala, Sweden

Abbreviations: GPMT = guinea pig maximization test; RAI = relative alkylating index.

the proteins, making them act as antigens. Some other metals such as platinum, palladium and rhodium, which are close to nickel, cobalt and chromium in the periodic table, also induce contact allergy, but they are not as common in the environment (Dupuis and Benezra, 1982).

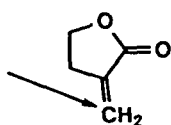
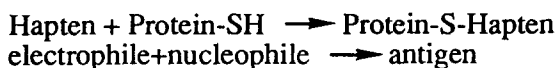
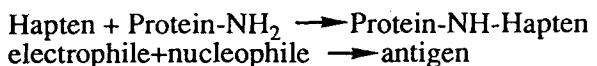
Methods of predicting sensitizing potential

An understanding of the chemical properties of a compound is essential to be able to predict the probability of that compound binding to proteins in the skin. However, other factors (e.g. the enzymatic

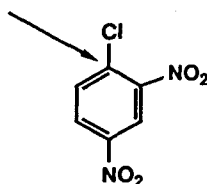
activity in the skin) are also important for the allergenicity of a substance. Predictions of sensitizing potential based on examining the chemical properties are therefore limited.

Since the reactivity between hapten and protein is considered important for the sensitizing property, the relation between the relative alkylating index (RAI) and the sensitizing potential of different compounds has been investigated in several studies (Betso *et al.*, 1991; Goodwin and Roberts, 1986; Roberts, 1987; Roberts *et al.*, 1991; Roberts and Williams, 1982). However, a correlation between RAI and sensitizing potential has

A

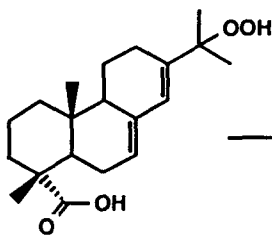
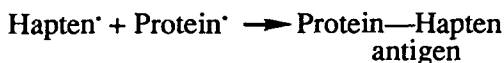


Tulipalin A
(in tulip bulbs)



2,4-Dinitrochlorobenzene
(DNCB)

B



15 - Hydroperoxyabietyl acid
(in rosin)

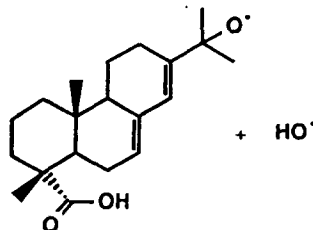


Fig. 1. Electrophilic properties are essential for the ability of a compound (hapten) to bind to skin proteins to create antigens, since the proteins are nucleophiles (rich in electrons). The protein bond can, however, also be formed by way of a radical mechanism. (A) Example of electrophilic haptens. The arrows indicate where the nucleophilic attack is likely to occur. (B) Example of a hapten able to bind to the protein by way of a radical mechanism.

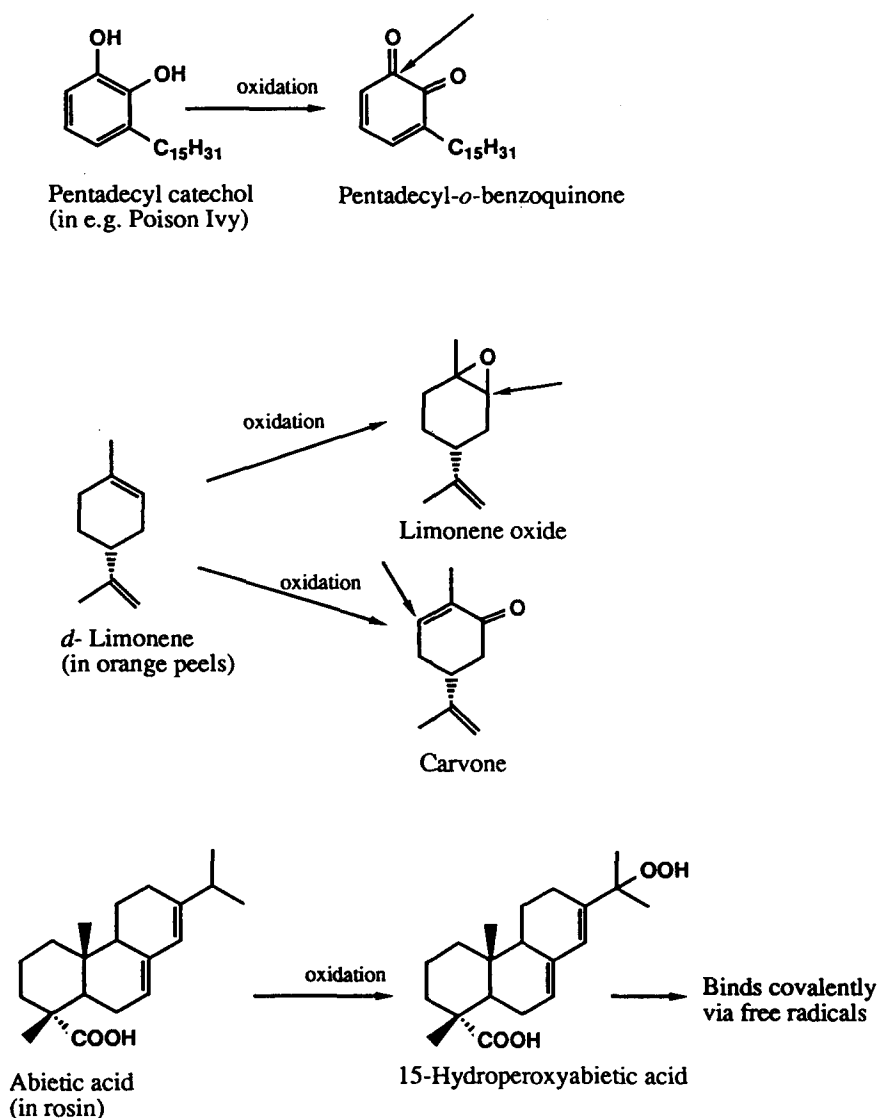


Fig. 2. Enzymatic processes in the skin or chemical transformation outside the skin can change the structure and make a substance (prohapten) allergenic that is not originally so. The figure shows transformation from a prohapten to a hapten through oxidation.

been obtained only for homologous series of molecules.

Attempts have been made to obtain a systematic overview regarding the correlation between structure and allergenic activity by creating a databank of all reported cases of allergic contact dermatitis as well as data from experimental sensitization studies (Benezra *et al.*, 1985). This can be helpful, provided that the collected reports are evaluated carefully. Otherwise a false and misleading picture of the allergenic activity of a compound may result, since not all reports are of equally high scientific quality.

The development of *in vitro* methods as alternatives to animal testing for predicting sensitizing capacity has not yet been successful. *In vivo* methods use either mice or guinea pigs, and the latter are considered to give the most reliable results. Updated guidelines

from the OECD (1992) for predicting the sensitizing capacity of a new compound were adopted in 1992 (Table 1). Here one of two alternative tests in mice, the local lymph node assay (Kimber and Basketter, 1992) and the mouse ear swelling test method (Gad *et al.*, 1986; Thorne *et al.*, 1991) is recommended for initial screening. When allergenic activity is demonstrated by these methods the use of the compound in humans must be questioned. However, if the substance gives no signs of allergenic activity in mice, this observation must be confirmed by experiments using validated guinea pig methods, since experience with the mouse methods is still limited. Two guinea pig methods, the guinea pig maximization test (GPMT) and the Buehler test, are recommended, although approximately 15 different methods using guinea pigs have been described. The

Table 1. Methods of determining the contact-allergenic activity of a compound

1. Structural formula	Determination of the reactivity of a compound.
2. Literature studies	Physical/chemical properties, data concerning skin irritancy, information on compounds with a similar structure.
3. Sensitization studies in the mouse	Screening methods.
4. Sensitization studies in the guinea pig	Guinea pig maximization test, Buehler test. Methods of high predictive value.
5. Sensitization studies in human	Not ethically acceptable.
6. <i>In vitro</i> studies	No methods exist.

After OECD Guideline for Testing of Chemicals. Skin sensitisation. (OECD, 1992).

GPMT method is the most widely used and best validated of these (Andersen and Maibach, 1985).

Conclusion

A compound containing electrophilic sites or one that can easily form free radicals has a potential to cause contact allergy, since it can form covalent bonds to proteins in the skin and create complete antigens. Also, compounds that are readily activated, for example by autoxidation, to form haptens must be taken into consideration. The indications must be confirmed experimentally in animals because no *in vitro* methods yet exist.

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