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An α,β-unsaturated oxime identified as a strong contact allergen Indications of antigen formation via several pathways

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Abstract

The aim of this study was to examine the possible skin sensitizing effect of oximes, employing an α,β-unsaturated oxime as the model compound. Oximes are not frequently used as biologically active compounds. However, they have been shown to possess both anti-inflammatory and anti-allergic activities. Furthermore, in a recent study, a number of oximes and oxime–ethers of hydroxylated benzaldehydes and acetophenones were found to be powerful antioxidants suggested to be used in consumer products such as cosmetics and food. Although there are only few reports on the sensitizing effect of oximes, their ability to be hydrolyzed to the corresponding ketones or aldehydes makes them potential contact allergens. The oxime investigated in this study was demonstrated to be a strong contact allergen in both mice and guinea pigs, capable of sensitize the control animals after only one dermal exposure. In order to elucidate the mechanisms for the formation of the complete antigen, a variety of analogues with different reactivity were tested. The results indicate that α,β-unsaturated oximes can react with proteins via several different pathways. Most likely, a metabolic transformation is involved. Due to the strong allergenic effect of the oxime investigated, we strongly advise against the use of such oximes in consumer products until a better understanding of their interactions with biological macromolecules has been obtained.

Keywords: Allergic contact dermatitis; Guinea pigs; Metabolism; Mice; Sensitization; Structure activity relationships

1. Introduction

Oximes are structural analogues of aldehydes or ketones formed by reaction of such compounds with hydroxylamine. They have a variety of industrial applications, e.g., as anti-skinning agents in paint, blocking agents in the polymer industry and chelators in the metal industry. Although not frequently employed as biologically active agents, it has been suggested that the anti-inflammatory and anti-allergic activities of oximes might be of clinical use (Katagi et al., 1992, 1996; Kataoka et al., 2002). Furthermore, a recent study revealed that a number of oximes and oxime–ethers of hydroxylated benzaldehydes and acetophenones are powerful antioxidants (Ley and Bertram, 2002). Most of them more potent in this respect than ascorbic acid, tocopherol and butylated hydroxytoluene, which are commonly used to prevent oxidation of various products during normal storage and handling. Consequently, oximes have been suggested to be used as antioxidants in

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consumer products such as, e.g., cosmetics and food (Ley and Bertram, 2002).

Oximes can readily be hydrolyzed to the corresponding ketones or aldehydes, which are chemically reactive electrophilic compounds and can react with nucleophilic groups in macromolecules in the skin, thereby producing complete antigens and inducing contact allergy. After prolonged or repeated exposure with the allergenic chemical, allergic contact dermatitis (ACD) will develop at the site of contact. ACD is a common health problem in Europe; 10–15% of the population are sensitized to one or more contact allergens (Nielsen et al., 2001). Once an individual has become sensitized the allergy remains throughout life. It is therefore of great importance to identify contact allergens and make appropriate risk assessments regarding their safe use before they are introduced on the market.

Although several aldehydes and ketones have been described as contact allergens (Roberts and Lepoittevin, 1998; Patlewicz et al., 2001), few reports on the sensitizing capacity of oximes are found in the literature. In a study from the 1980s (Gad, 1988), the sensitizing capacity of 2-butanone oxime was investigated in guinea pigs and mice where it was identified as a mild sensitizer. Since oximes can be metabolized into hydroxylamines, they are also included as a structural alert in the latest update of the DEREK rules (Deductive Estimation of Risk From Existing Knowledge) (Barratt and Langowski, 1999), a computer-based predictive system aiming to identify contact allergens from their chemical structure.

In a previous study we examined the mechanism for antigen formation of carvone (Fig. 1), a known contact allergen containing an α,β-unsaturated keto-function (Nilsson et al., 2001). Carvone was found to react preferentially via a Michael reaction after attack at the β-carbon. The double bond was crucial for the allergenic activity since a saturated analogue containing only the ketone function was found to be devoid of sensitizing capacity.

The aim of the present investigation was to further evaluate the effect of oximes as skin sensitizers, since conflicting information concerning their allergenic activity has been reported. Due to their ability to be transformed into their parent carbonyl compounds, we consider them to be potential contact allergens. Therefore, we synthesized an α,β-unsaturated oxime with a structure related to carvone (compound **1**, Fig. 1). Its sensitizing capacity was investigated in mice and guinea pigs, and its chemical reactivity towards sulphur and nitrogen containing compounds was investigated using thiophenol and benzylamine as model nucleophiles. In addition, a number of analogues (compounds **2, 3** and **4**, Fig. 1) with different chemical reactivities were synthesized and tested for sensitizing capacity and cross reactivity in guinea pigs.

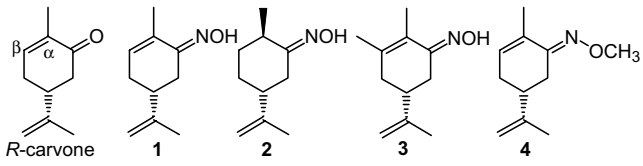

Fig. 1. Structures of compounds studied.

2. Material and methods

2.1. Chemicals

(5*R*)-5-Isopropenyl-2-methyl-2-cyclohexenone (*R*-carvone, 98%) was purchased from Aldrich Chemical (Stockholm, Sweden). Freund's complete adjuvant (FCA) and Freund's incomplete adjuvant (FIA) were obtained from Difco (Detroit, MI, USA). White non-stabilized petrolatum was obtained from VWR International (Stockholm, Sweden).

2.2. Instrumentation and mode of analysis

NMR spectroscopy was performed employing a JEOL Eclipse 400 instrument, using $CDCl_3$ solutions (residual $CHCl_3$ δ 7.26 and $CDCl_3$ δ 77.0 as internal standards). Electron-ionization mass spectral analysis (70 eV) was performed on a Hewlett–Packard model 5973 mass spectrometer connected to a gas chromatograph (GC; Hewlett–Packard model 6890). This GC was equipped with a cool on-column capillary inlet and an HP-1MS fused silica capillary column (30 m × 0.25 mm, 0.25 μm, Agilent Technologies, Palo Alto, CA, USA). The temperature program started at 35 °C for 1 min and increased thereafter with 10 °C/min until a final temperature of 250 °C was reached and maintained for 2 min. Helium was used as the carrier gas and the flow-rate was 1.2 mL/min. Optical rotations were measured at ambient temperature using a Perkin–Elmer 241 polarimeter. Silica gel column chromatography was performed using Kieselgel 60 (230–400 mesh).

2.3. Synthesis

2.3.1. (5R)-5-Isopropenyl-2-methyl-2-cyclohexene-1-one oxime (*1*) (Scheme 1)

The synthesis was performed as described in the literature (Tkachev et al., 1995) producing **1** (82% yield) with ^1H and ^{13}C NMR spectral data corresponding to literature data (Sivasubramanian et al., 1984; Tkachev et al., 1995). MS 165 (M^+ 59%), 150 (28%), 148 (99%), 132 (47%), 123 (81%), 118 (27%), 107 (100%), 91 (67%); mp 72 °C (lit. 72 °C).