A Study of the Phototoxicity of Lemon Oil

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Summary. Lemon oil contains furocoumarin derivatives and is known to cause phototoxicity. In this study, lemon oil was fractionated, and its phototoxic activity was measured by means of a biological assay. The substances producing phototoxicity were identified by high-performance liquid chromatography as being oxypeucedanin and bergapten. The phototoxic potency of oxypeucedanin was only one-quarter of that of bergapten. However, the amounts of these two phototoxic compounds present in lemon oils produced in different regions of the world varied by a factor of more than 20 (bergapten, 4--87 ppm; oxypeucedanin, 26--728 ppm), and their ratio was not constant. The two compounds accounted for essentially all of the phototoxic activity of all lemon-oil samples. Among various other citrus-essential oils investigated, lime oil and bitter-orange oil also contained large amounts of oxypeucedanin. Oxypeucedanin was found to elicit photopigmentation on colored-guinea-pig skin without preceding visible erythema.

Key words: Phototoxicity - Lemon oil - Photopigmentation - Oxypeucedanin - Furocoumarin

Introduction

It has been reported that lemon oil elicits a phototoxic reaction in animal skin [2]. Various coumarin and furocoumarin derivatives are present in plants of Umbelliferae [4, 15], and some of these are known to be phototoxic. However, the components responsible for the phototoxicity of lemon oil have not previously been identified. Lemon oil, which is a very popular fragrance among the Japanese, also has a photopigmentation activity which may be important because of the high tanning ability of Japanese skin.

Materials and Methods

Animal Tests for Phototoxicity

Hartley-strain albino guinea pigs weighing 450--550 g were used for the evaluation of phototoxicity. Their dorsal area was shaved and depilated 24 h before exposure. After placing the animals on their stomach in animal holders, a solution (0.02 ml) of the test material in 99.5% ethanol or hexane was topically applied to two sites (concentration, 1 g per 100 ml solvent). One of these sites was covered with aluminum foil; the other was exposed to UV irradiation (320--400 nm) from six fluorescent lamps (Toshiba 40-BLB fluorescent lamp) through a glass filter excluding wavelengths of below 320 nm. The distance between the lamps and the skin was 10 cm. The total energy of the radiated ultraviolet light over 110 min was 13 J/cm² as measured using a thermopile. After irradiation, the animals were returned to individual cages, and their skin reaction was assessed 24, 48, and 72 h after exposure according to the following criteria: 0, no erythema; 1, very slight erythema; 2, well-defined erythema; 3, moderate to severe erythema; 4, severe erythema and edema.

Animal Tests for Photopigmentation

Weiser-Maple-strain colored guinea pigs weighing 500--600 g were used for the evaluation of photopigmentation. Prior to exposure, they were shaved with an electric shaver. The procedure was the same as that described for the phototoxicity tests. Skin reactions were examined 24, 48, and 72 h after exposure, and pigmentation was evaluated according to the following criteria: 0, no pigmentation; 1, very slight pigmentation; 2, well-defined pigmentation; 3, moderate to severe pigmentation; 4, severe pigmentation.

Fractionation of Lemon Oil

Using vacuum distillation under 1 mm Hg at 95°C, the lemon oil (5 kg) was first fractionated into two fractions -- the distilled fraction and the residue fraction (Fig. 1). The residue was partitioned between 600 ml methanol and 1,200 ml hexane, and...
both phases were then concentrated. The methanol fraction was subjected to silica-gel column chromatography in the usual way. The solvent systems used were mixtures of 10% – 50% ethyl acetate in hexane. Each fraction obtained by this procedure was evaluated for phototoxicity and analyzed for the presence of bergapten using high-performance liquid chromatography (HPLC). The fraction that produced a phototoxic reaction was further separated by silica-gel column chromatography. Finally, two compounds were crystallized from the F-3-B fraction (see Fig. 1). These were confirmed to be pure by HPLC and thin-layer chromatography, and were identified using nuclear magnetic resonance (NMR), mass spectral analyses, and infrared spectrum (IR).

**High-Performance Liquid Chromatography**

The high-performance liquid chromatograph consisted of a 6,000 A pump (Waters), a UV detector set at 310 nm, and a fluorescence detector (exciting wavelength, 365 nm; emission wavelength, 485 nm). For bergapten determination, three columns of Silicapak E-411 (Showa Denko; length, 15 cm) were used, with dichloromethane as the eluant; for oxypeucedanin determination, two columns of μ-Porasil (Waters) were used, with hexane-isopropanol (100:1) as the eluant (flow rate, 2.5 ml/min).

**Nuclear Magnetic Resonance**

NMR spectra were recorded on an FX-90Q spectrometer (Nippon Denshi). Samples were run in deuterated chloroform, using tetramethylsilane (TMS) as an internal reference.

**Materials**

The bergapten used as a standard compound was isolated and purified from bergamot oil. Bergapten was recrystallized three times from a 99.5% alcoholic solution of the fraction, which was distilled under 4 mm Hg at 220°C – 260°C from bergamot oil [8].

**Results**

**Isolation and Identification of Phototoxic Compounds**

The results of the biological assay for the phototoxicity of each fraction obtained from lemon oil are shown in Table 1. The test concentration of each fraction was adjusted with solvent to correspond to the original concentration in lemon oil. Phototoxic