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PHOTODYNAMIC REACTIONS INDUCED BY COMPOUNDS DERIVED FROM LICHENS

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Abstract. Contact dermatitis from lichens is now well documented but the possible influence of exposure to sunlight is less clear. Positive reactions on photopatch testing has recently been described, but whether this represented an unspecific exacerbation or a true photoallergic response was difficult to evaluate. In this study 13 different substances derived from lichens commonly found in nature were investigated with regard to their capacity to induce photo-oxidative membrane damage, as revealed by the photohemolysis technique. It was found that the earlier suggested ability to induce photosensitization could be confirmed for several of the lichen compounds investigated. It was also shown that singlet state excited oxygen may participate to some degree in some of these reactions.

Key words: Photosensitization; Photohemolysis; Lichens

Lichens are plants composed of fungi living in symbiosis with algae. The fungi obtain the products of photosynthesis from the algae and contribute in return minerals and protection from exposure to high-intensity solar radiation (1, 2, 5, 7, 10).

Allergic skin reactions with contact dermatitis have been reported only rarely but it is now a well documented phenomenon (3, 4, 10, 11, 12, 13). Lichens can be found on a wide variety of trees, rocks and in soils and contact dermatitis is primarily seen following forestry or horticultural work.

Some patients have noted exacerbations from exposure to sunlight (17, 18) and the role of solar radiation has therefore earlier been discussed (10, 18). Whether this may be due to an unspecific exacerbation caused by sunlight, or a manifestation of a photosensitizing capacity from lichens, is not yet clear. However, many lichens fluoresce under longwave ultraviolet irradiation, indicating that they may exert or participate in photodynamic reactions.

Patients with a previous history of photosensitivity to longwave ultraviolet light have recently been reported to react with contact dermatitis to lichens, but photopatch testing induced even stronger reactions, suggesting that lichens can cause both contact and photocontact dermatitis (18).

In the present paper some compounds derived from lichens commonly found in nature have been investigated with regard to their capacity to induce photo-oxidative membrane damage, as revealed by the photohemolysis technique.

MATERIAL AND METHODS

Photohemolysis

The modified technique of Kahn & Fleischaker (6) earlier used by us (16) for investigation of photosensitizing compounds was applied. Erythrocytes were obtained from healthy human adults.

The investigated substances were 13 different oleoresins derived from lichens; Alantolactone, atranorin, barbatic acid, evernic acid, fumarprotocetraric acid, gyrophoric acid, lecanoric acid, lobaric acid, psoromic acid, salazinic acid, stictic acid, usnic acid and physodalic acid. Alantolactone and usnic acid were obtained from Fluka AG, Chemische Fabrik, CH-9470 Buchs, Switzerland. The other substances were kindly isolated by Dr Y. J. Solberg, Chemical Research Laboratory, Agricultural University of Norway, by the use of earlier described techniques (14, 15). All substances used in the experiments were kindly donated by Dr Per Thune, Department of Dermatology, Rikshospitalet, Oslo, Norway.

Absorption spectra were established but no other attempt was performed to further analyse the purity of the extracts. The compounds were dissolved in DMSO, giving a final maximum concentration of 0.2–0.02% DMSO, and in 0.1 M Na-Veronal (barbital) buffer at pH 8.0. The substances were tested at a concentration of 1, 10 and 25 mg per 100 ml, but atranorin also at 50 and 100 mg per 100 ml. No correction was performed with regard to molecular weight.

Packed human red blood cells were washed three times in physiological saline and 0.1 ml was then added to 10 ml of the buffered solutions of the substances mentioned and to control solutions which were poured into quartz cuvettes. Controls were incubated in the dark. As a further internal comparative control, kynurenic acid, a potent photosensitizer capable of singlet oxygen mediated membrane damage, was included and tested at a concentration

Table 1. *Photohemolysis induced by oleoresins derived from lichens, expressed in per cent for different concentrations of the test substance with or without D_2O as solvent instead of H_2O (deuterium test)*

Absorption maxima are seen in the first column when registered in the 280–600 nm region. SH indicates spontaneous hemolysis without irradiation

		Photohemolysis %					
	Absorption maxima,	H_2O			D_2O		
Oleoresins investigated	280–600 nm	25 mg %	10 mg %	1 mg %	25 mg %	10 mg %	1 mg %
Alantolactone	0	SH	SH	0	SH	SH	0
Atranorin[1]	282 388	8	0	0	0	0	0
Barbatic acid	288 306	SH	31	3	SH	SH	0
Evernic acid	300	23	0	0	18	3	0
Fumarprotocetraric acid	320	SH	SH	0	SH	SH	0
Gyrophoric acid	302	15	4	0	28	22	7
Lecanoric acid	304	7	0	0	17	7	0
Lobaric acid	290	SH	SH	0	SH	SH	31
Psoromic acid	316	28	33	3	–	39	5
Salazinic acid	314	SH	12	0	SH	SH	0
Stictic acid	312	14	0	0	3	0	0
Usnic acid	290	0	0	0	0	0	–
Physodalic acid	310	SH	SH	0	SH	SH	0
Internal standard for comparison:							
Kynurenic acid	335		48			71	

[1] Atranorin was further tested in higher concentrations and gave 14 and 22 % hemolysis in the concentrations 50 and 100 mg per 100 ml, respectively.

of 10 mg per 100 ml. All experiments were performed several times.

Test solutions were exposed to an Osram High Pressure Xenon arc lamp (XBO 150 W) at a distance of 40 cm from the lamp. In order to preclude shortwave ultraviolet radiation (UVB and UVC) the lamp was equipped with a filter combination of a Schott WG 295 and a KG 1 heat-protective filter, which was found more effective than the use of a 3 mm ordinary glass filter. The intensity of the lamp in the UVA region as measured primarily around the 360 nm band with a Waldmann UVA-meter (H. Waldmann Werk für Lichttechnik, Germany) was 13 mW/cm². The UVA dose given in all experiments was fairly high, 15 Joule/cm².

After irradiation, the test suspensions were centrifuged at 2 000 rpm and the optical density of the supernatant fluid was read at 540 nm on a Beckman DB spectrophotometer and compared with a total hemolysis control and with the dark control. Results were expressed in percent relative to the 100 % hemolysed solution (6, 16).

The deuterium test for identification of singlet oxygen mediated reactions has earlier been described in detail (9, 16). It was performed for all substances.

Absorption spectra

Absorption spectra were recorded with a Beckman DB Spectrophotometer for all substances dissolved in ethanol. Plausible absorption was investigated and followed from 600 nm downwards through the UVA and UVB wavelengths range. For substances providing absorption within these regions the maximum values were registered and are indicated in Table 1.

RESULTS

In the control experiments with erythrocytes irradiated without the presence of lichen compounds, no photohemolysis at all was seen in spite of the high UVA dose of 15 Joule/cm², but also irrespective of whether DMSO was added or not. Control experiments with the photosensitizer kynurenic acid showed that the addition of DMSO did not influence the photohemolytic reaction.

Several oleoresins were extremely toxic to the red blood cells, viz. alantolactone, barbatic acid, fumarprotocetraric acid, lobaric acid, salazinic acid and physodalic acid, resulting in spontaneous hemolysis without irradiation for the higher concentrations tested and could therefore not be further tested (Table 1).

As seen in Table 1, significant photohemolysis was induced by all but four substances, viz. alantolactone, fumarprotocetraric acid, usnic acid and physodalic acid. However, these compounds showing negative results (except usnic acid) could

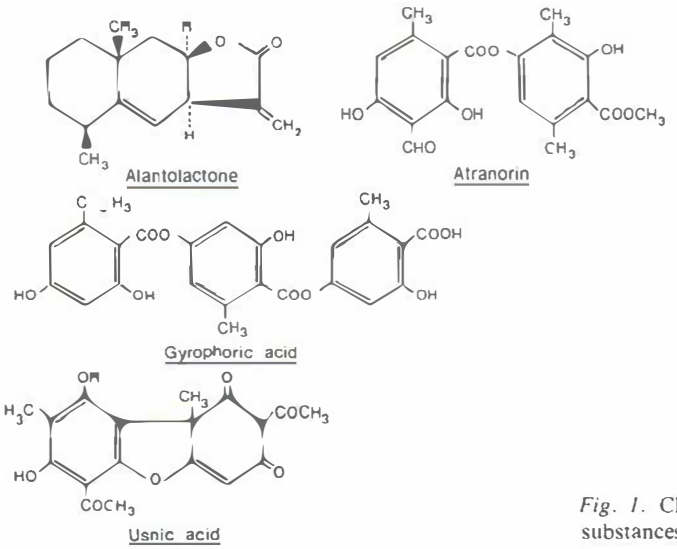

Fig. 1. Chemical formula for some of the investigated substances derived from lichens.

not be tested at higher concentrations as they then caused spontaneous hemolysis. The other substances induced a varying degree of photohemolysis ranging up to 39%. The reaction was sometimes reinforced by using D_2O as solvent instead of H_2O—the deuterium test—as seen for gyrophoric acid, lecanoric acid, and lobaric acid, indicating that singlet oxygen mediated mechanisms may at least partly be involved in the reactions.

For comparison, the photohemolysis induced by the potent photosensitizer kynurenic acid at a concentration of 10 mg per 100 ml (16) under the present experimental conditions used is included in Table 1.

DISCUSSION

Lichens are commonly found in nature and sensitization may happen principally during forestry work. However, even in an urban environment, contact with lichens is quite conceivable.

Some 17 000 species of lichens are known, capable of synthesizing many different chemical substances which accumulate and may represent up to 5% of their dry weight, such as usnic acid. Usnic acid is one of a group of coloured lichenic acids, a monobasic acid with dibenzofuran structure and antibiotic properties. Dibenzofuran is chemically related to the furocoumarans.

Lichens are common in temperate zone forests and allergy to them seems to be more common than is presently recognized. Large quantities may be liberated into the air when lichens are fragmented, as for example when old wood is handled and especially during dry seasons and in sunny weather. Lichen allergy is unlikely to be as frequent in urban as in rural life. However, with increasing leisure time and the present trend towards more outdoor life during vacations, these problems may afflict even urban citizens. Lichens are fairly common in Scandinavia.

Contact dermatitis from lichens is now well documented but the possible influence of exposure to sunlight is less clear. Airborne contact dermatitis simulating photodermatitis has been proposed to explain the clinical features seen in patients with lichen allergy. However, relapses on sun exposure have been reported by several patients (17). Photopatch testing, indicating the possibility of photosensitization with occurrence of photoallergic contact dermatitis, has recently been described (18). Contact dermatitis but an even stronger reaction in irradiated patches provoked by UVA-radiation was seen. Whether this reaction represents un unspecific exacerbation or a true photoallergic response was difficult to evaluate.

Photohemolysis is a suitable semiquantitative method (6, 16) for screening of suspected photosensitizers capable of photo-oxidative reactions. Substances which do not require molecular oxygen for their action, such as furocoumarans, give no photohemolysis and the same is true of chemicals inflicting damage to other cellular organelles which

erythrocytes are devoid of. Thus, some potent photosensitizers do not always induce photohemolysis. Therefore, negative reactions require further investigations.

In the present experiments a more or less pronounced photohemolysis was provided by nine of thirteen investigated substances derived from lichens. Four gave quite negative results but three of them could not be further tested in higher concentrations due to pronounced spontaneous hemolysis, viz. alantolactone, fumarprotocetraric acid and physodalic acid (Table I). These substances should be investigated by other methods, but alantolactone showing no absorption in the 280–600 nm region is probably not a photosensitizer. Usnic acid does show absorption commencing at 380 nm and below, with its maximum at 290 nm. Many lichen derivatives show fluorescence on UVA irradiation, indicating that they may participate in photochemical reactions, but usnic acid does not (10) and is furthermore not capable of inducing photohemolysis.

The remaining substances all induced photohemolysis to a varying degree, most pronounced for barbatic acid, evernic acid, gyrophoric acid, lobaric and psoromic acid. However, it must be remembered that the method is semiquantitative and furthermore that the concentrations used are not quite comparable on a molecular basis. Thus, which of the investigated substances have the greatest photosensitizing capacity cannot be stated with certainty. Nevertheless, the photohemolytical technique is a valuable method for a preliminary screening of suspected photosensitizers from lichens or plants in order to determine which should be used in photopatch testing procedures on patients or which substances need to be further investigated by other methods.

In this study the earlier suggested capacity of lichen derivatives to induce photosensitization was confirmed for several of those investigated. It was also shown that singlet state excited oxygen may participate to some degree in some of these reactions.

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