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Experimental Contribution to the Dithranol-Brown Problem

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Dithranol is well known to dermatologists: during antipsoriatic therapy with dithranol, a brownish staining frequently occurs in the lesions as well as the surrounding non-involved skin, and there have been various efforts [5] to eliminate or to diminish this side effect. The present study is concerned with dithranol brown located in the brown psoriatic scales obtained from patients undergoing dithranol therapy (dithranol with 2% salicylic acid in white soft paraffin).

Microscopic examination showed that dithranol brown is deposited in the form of well-defined brownish particles in the stratum corneum. These particles were isolated using a micromanipulator and introduced into a 311A mass spectrometer via a heated inlet system (90° C, 120° C). High resolution revealed the presence of hydrocarbons [$C_{18}H_{36}$, $C_{28}H_{56}$, $C_{27}H_{44}$ (cholestadiene)] and an oxygenated steroid $C_{27}H_{46}O$ (cholestenol or cholestanone). When the inlet system of a CH5 mass spectrometer was heated to 450° C, we obtained a very weak signal at m/z = 446 from a dimethylsulfoxide (DMSO) extract of brown scales, which might be attributable to tetrahydroxyhelianthrone [2]; under these conditions, however, the formation of artifacts cannot be excluded.

The proteolytic degradation of brown scales using Pronase followed by $CHCl_3$ extraction revealed the molecular ions of dithranol, chrysazine and 1,8,1′,8′-tetrahydroxybisanthrone (m/z = 450; <1%), and the corresponding fragment ions (Fig. 1).

The excitation of a single brown particle to an ionized state under microscopic control using a focused laser beam (LAMMA [3]) indicated the presence of copper (m/z = 63 and m/z = 65; intensity relation approximately 2:1); under identical conditions, no copper was found in the surrounding light-coloured area. The application of LAMMA to scales from psoriatic lesions which had been treated with dithranol revealed the presence of negative ions at 240 mu (chrysazine) and 225 mu [either $(M-1)^-$ of dithranol or a fragment ion of 1,8,1′,8′-tetrahydroxybisanthrone; bisbenzylic cleavage]. The validation of these results with dithranol, chrysazine and the bisanthrone indicated m/z = 225 for dithranol, $[(M-H)^-]$ and for the bisanthrone (fragmentation of the bisbenzylic bond), while chrysazine produced $M^{\cdot -}$ at m/z = 240, followed by loss of 29 mu ($HCO^{\cdot}$) to m/z = 211 (Fig. 2) [6].

When we heated brown scales to 300° C in a TAS apparatus (DESAGA, Heidelberg, FRG), we obtained a yellow sublimate which contained chrysazine. Van Duuren et al. [8] have prepared complexes of chrysazine with various metals including copper. Although copper and chrysazine are found in the same compartment, the identity of Van Duuren's complex and dithranol brown cannot be deduced from

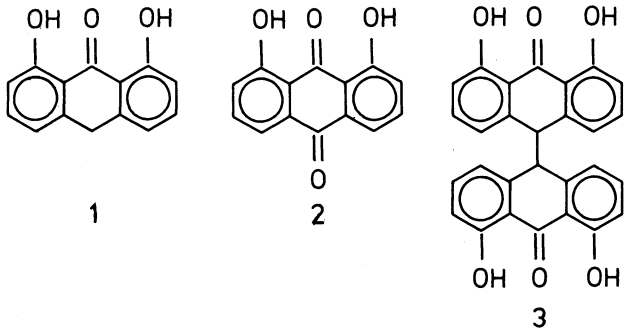

Fig. 1. Formulas of dithranol (*1*), chrysazine (*2*) and 1,8,1′,8′-tetrahydroxybisanthrone (*3*)

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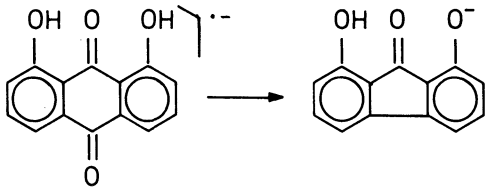

Fig. 2. LAMMA of chrysazine: loss of HCO·

Table 1. Penetration und metabolization of 1% dithranol in white soft paraffin in psoriatic patients in vivo. Separation of the skin by suction blister technique

	Penetration time [min]	Epidermis	Blister fluid	Dermis
Psoriatic lesion	60	*1*: + *2*: +++ *3*: ++	*2*: +	Negative
Uninvolved skin	285	*2*: +	*2*: +	*2*: +

this experiment. We were unable to isolate this complex or any similar copper complex from the brown scales; Van Duuren's statement about the solubility and ligand exchange of his complex explains this failure. Our results, however, pointed towards a metabolization of dithranol to chrysazine in the epidermis. This proved to be true: after being treated with dithranol in white soft paraffin, the epidermis and dermis of the involved and uninvolved skin of psoriatic patients were separated by suction blisters [4]. The epidermis, the blister fluid and the upper part of the dermis were analysed (Table 1).

Although Sa e Melo et al. [7] using gas chromatography mass spectrometry (GC-MS) after the topical application of dithranol to normal human skin did not detect dithranol or its metabolites as silylized derivatives in suction-blister fluid, our results indicate that chrysazine and 1,8,1′,8′-tetrahydroxybisanthrone occur in the blister tissues of psoriatic skin. Although unlikely, the contribution of melanin to the discolouration was considered, because tyrosinase (polyphenol oxidase) is a Cu-protein. This consideration was experimentally cancelled: the hypomelanotic areas of vitiligo patients are stained by dithranol.

Experiments

1. Isolation of Brown Particles from Scales

Brown particles were excised out of brown scales using a micromanipulator under microscopic control. Brown particles (0.1 mg) were extracted with DMSO in a Soxhlet apparatus, the solvent was evaporated in vacuo and the residue was analysed by mass spectrometry.

Table 2. High resolution measurements

m/z	Formula	Calculated	Found
252	$C_{18}H_{36}$	252.28169	252.28118
368	$C_{27}H_{44}$	368.34429	368.34443
386	$C_{28}H_{50}$ (65%)	386.39123	386.39013
386	$C_{27}H_{46}O$ (35%)	386.35485	386.35470

Brown scales (15 mg) were degraded with 10 mg Pronase (Boehringer) in 10 ml Tris buffer for 2 h at 25° C. The mixture was extracted three times with 10 ml $CHCl_3$, and the organic solution was dried (Na_2SO_4), evaporated in vacuo and analysed by thin layer chromatography (tlc) (Al sheets, silica 60 F_{254}, toluene/glacial acetic acid 80/20 v/v) and mass spectrometry. The high-resolution measurements (311 A) of the extract are given in Table 2.

2. Electron-Impact (EI) Mass Spectrometry

The mass spectra of dithranol, chrysazine and 1,8,1′,8′-tetrahydroxybisanthrone (CH 5) are as follows:

1. Dithranol: 12eV: m/z = 226 ($M^{+\cdot}$, 100%); 70eV: m/z = 226 (100%), 198 ($M^{+\cdot}-CO$, 22%), 181 (8%)

2. Chrysazine: 12eV: m/z = 240 ($M^{+\cdot}$, 100%); 70eV: m/z = 240 (100%), 212 ($M^{+\cdot}-CO$; 14%; m* = 187.27), 184 (212−CO; 10%, m* = 159.70)

3. 1,8,1′,8′-tetrahydroxybisanthrone: 11eV: m/z = 450 ($M^{+\cdot}$, <1%), 225 (100%); 70eV: m/z = 225 (100%), 197 (225−CO; 48%)

3. Laser Microprobe Mass Analyser (LAMMA) Experiments

All measurements with $\lambda_{Laser} = 265$ nm; $\tau_{Laser} = 10$ ns. The positive mass spectra of the brown particle revealed m/z = 63^+ (Cu, 100%) and 65^+ (Cu, 50%). The negative mass spectra of dithranol indicated m/z = 225 [(M-H)$^-$, 100%]. One day after preparation, the pertinent spectra showed: m/z = 225 [(M-H)$^-$, 100%], 240 (chrysazine$^{-\cdot}$, 43%) and 211 (5.5%). Under identical conditions, chrysazine led to fragments at: m/z = 240 ($M^{-\cdot}$, 100%) and 211 [(M-HCO·)$^-$, 80%] while 1,8,1′,8′-tetrahydroxybisanthrone was cleaved to m/z = 225 ($M/2^-$, 100%).

4. Preparation of the Chrysazine-Cu Complex

This was performed according to the method of Van Duuren et al. [8].

5. Pyrolysis of Brown Scales in a TAS Apparatus

About 10 mg brown scales were cut into pieces, placed in the glass tube of a TAS apparatus and heated to 150°, 200°, 250°, 300° and 350° C.

6. Analysis of Blister Tissue

Highly refined 1% dithranol [1] in white soft paraffin was applied to both the uninvolved skin and lesions of psoriatic patients in vivo; after defined periods, it was removed by washing with a hydrophile lipogel (Table 1). The epidermis and dermis were separated using the suction-blister technique [4], and the tissue samples were stored at −25° C. Histologically identical samples were combined in batches of 2−30 mg and extracted with 5 ml acetone under N_2 at room temperature. The solvent was evaporated in a N_2 stream, and the residue was dissolved in 0.50 ml acetone; 10 µl of this solution were used for tlc.

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References

1. Auterhoff H, Scherff FC (1960) Die Dianthrone der pharmazeutisch interessierenden Hydroxyanthrachinone. Arch Pharm (Weinheim) 293:918−925
2. Brockmann H, Neef R, Mühlmann E (1950) Zur Synthese hypericin-ähnlicher Oxy-naphthodianthrone. Chem Ber 83:467−484
3. Hillenkamp F (1983) Laser-induced ion formation from organic solids. Springer Ser Chem Phys 25:190−205
4. Kiistala U, Mustakallio KK (1964) In vivo separation of epidermis by production of suction blisters. Lancet 1:1444−1445
5. Runne U, Kunze J (1982) Short-duration ("minutes") therapy with dithranol for psoriasis: a new out-patient regimen. Br J Dermatol 106:135−139
6. Runne U, Rosmarinowski J, Safar GJ, Kupka KD, Schultz-Amling W, Plumier E, Wiegrebe W (1983) Demonstration of dithranol in normal and psoriatic skin with laser-microprobe mass analysis (LAMMA) and biochemical techniques. Measurement methods for the in vivo penetration and metabolization. Arch Dermatol Res 275:269−270
7. Sa e Melo T, Dubertret L, Prognon P, Gond A, Mahuzier G, Sautus R (1983) Physiochemical properties and stability of anthralin in model systems and human skin. Invest Dermatol 80:1−6
8. Van Duuren BL, Segal A, Tseng S-S, Rusch GM, Loewengart UM, Roth D, Smith A, Melchionne S, Seidman J (1978) Structure and tumor-promoting activity of analogues of anthralin. J Med Chem 21:26−31

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