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REPORTS

# Physicochemical Properties and Stability of Anthralin in Model Systems and Human Skin

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**The physico-chemical properties and the stability of anthralin, a potent antipsoriatic agent, has been investigated in model systems by optical absorption and fluorescence spectroscopy and by gas chromatography coupled to mass spectrometry. Systematic studies were carried out on anthralin and its oxidation products (1,8-dihydroxyanthraquinone and 1,8-1′,8′-tetrahydroxydianthron). Anthralin and 1,8-dihydroxyanthraquinone are shown to readily bind to human serum albumin and not to DNA. Anthralin bound to albumin readily oxidizes, yielding the 1,8-dihydroxyanthraquinone which is fairly stable. These results are correlated with those obtained with intact whole human epidermis and suction blister fluid showing that, in the former case, anthralin binds to protein as suggested by absorption and fluorescence spectroscopies. Gas chromatography–mass spectrometry analysis makes it easy to detect anthralin and 1,8-dihydroxyanthraquinone in suction blister fluid doped with anthralin but not in suction blister obtained after topical application on normal human skin.**

Anthralin is largely used in dermatology as a potent antipsoriatic drug [1, 2]. However anthralin is a very unstable product easily oxidized to numerous compounds [3,4]. Thus, it is actually uncertain whether the molecular events leading to its therapeutic efficiency are linked to anthralin itself or to breakdown oxidation products liberated into the skin. For example, it has been recently shown that the so-called "anthralin brown" inhibits glucose-6-phosphate dehydrogenase (G6PDH), an enzyme which is thought to be involved to some extent in the abnormal cell proliferation characterizing psoriasis [5, 6]. Moreover, most biological studies on anthralin were performed under physicochemical conditions in which anthralin is quickly destroyed and, as a consequence, the results are difficult to interpret.

The aim of this study was to investigate the physicochemical properties of anthralin and its oxidation products in model media by absorption and fluorescence spectroscopies and gas chromatography coupled to mass spectrometry (GCMS) in order to obtain more information required by future biological studies.

On the basis of the information gathered in model systems, the stability of anthralin was studied in whole human epidermis and in the interstitial fluid after *in vitro* incubation and *in vivo* topical application.

## EXPERIMENTAL

Chemicals of the purest commercially available grade were used as received. Purified anthralin (I), 1,8-dihydroxyanthraquinone (II) and 1,8-1′,8′-tetrahydroxydianthron (III) were a gift of Dr. B. Shrott (C.I.R. D. 06 Valbonne, France). They were kept frozen (−22°C) in the dark. Absorption spectra in ethanol were taken from time to time in order to check their stability. Tris and phosphate buffers at various ionic strengths and pH were prepared in triply distilled water. Solvents such as ethanol, acetone, chloroform, and dimethylformamide were Merck UVASOL grade products. Bovine and human serum albumins (BSA or HSA) were supplied by Sigma as well as DNA from calf-thymus.

Optical spectra on the skin samples were recorded with a Cary 219 spectrophotometer while fluorescence measurements were performed with either an Aminco-Bowman spectrofluorophosphorimeter or a FICA 50 M fluorimeter equipped with excitation and emission spectrum corrections. When anaerobic conditions were required, spectrophotometry was performed in solutions bubbled with argon or nitrogen provided by Air Liquide.

Intact human skin samples (~ 2 $cm^2$) were obtained from blisters induced by mild suction as already described [7]. They were kept in Hanks' culture medium devoid of phenol red. Skin samples were obtained from areas that had been treated one day before spectrophotometric analysis with anthralin (ointment or 0.5% solutions) as specified in some figure legends. Untreated skin samples were, in some cases, incubated in the dark with anthralin in Hanks' medium. In this case, the incubation medium was prepared by adding up to 2% of a $10^{-2}$ M stock anthralin solution in either ethanol or acetone to the desired volume of Hanks' medium. Incubation times were up to 3 hr. Because of anthralin instability, solutions were handled in the dark and kept under nitrogen.

The skin samples thus obtained have a good light transmission up to ~ 350 nm [8] so that optical absorption spectroscopy can be directly performed on human epidermis. In this regard, 6-mm diameter circular skin patches (thickness 50 μm) were cut with a puncher and placed into 0.1 mm optical quartz cells. In order to obtain good base lines an untreated blank skin sample prepared in the same way was intercalated into the reference beam of the spectrophotometer.

The blister fluid which is a good representative of the interstitial fluid [9] was analyzed by optical spectroscopy and gas chromatography coupled to mass spectrometry (GCMS). A Hewlett Packard type HP 5992 B Mass spectrometer coupled

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Abbreviations:
BSTFA: bistrimethylsilyl trifluoroacetamine
DMF: dimethylformamide
GCMS: gas chromatography coupled to mass spectrometry
G6PDH: glucose-6-phosphate dehydrogenase
SIM: single ion monitoring technique
TMCS: trimethylchlorosilane

with a gas chromatographer equipped with a 25 m × 0.3 mm i.d. glass capillary column coated with SE-30 was used. Helium filtered on molecular sieve was used as a carrier gas at a flow rate of 2 ml/min. Instrument temperatures were—injector: 189°C—oven from 180° to 280°C (with temperature programming: 6° per min). The mass spectrometer was used under the following conditions: accelerating voltage 1800 V, ionization energy 70 ev.

Biological materials were first extracted by acetone and then by benzene. After evaporation of the solvent under a nitrogen stream, residues were silylated with a mixture of bistrimethylsilyl trifluoroacetamine (BSTFA) and trimethylchlorosilane (TMCS) (80/20 v/v), in a screw-capped septum vial, heated at 80°C in the dark for 15 min [10].

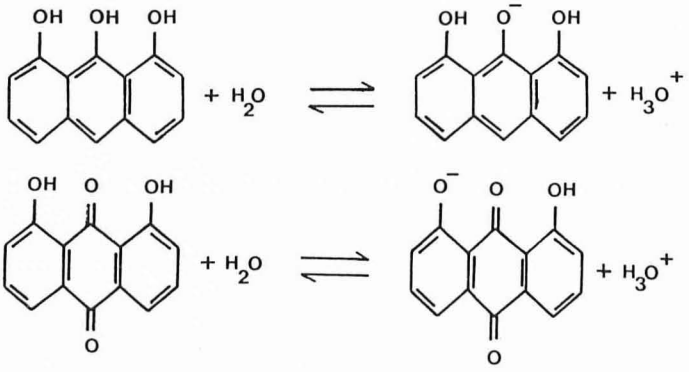
## RESULTS

### *Optical Absorption Spectroscopy in Model Systems*

A characterization of anthralin and derivatives in human skin by optical absorption spectroscopy requires the knowledge of the solvent effect and thus a study of the polarity, pH and hydrogen bonding effects on the anthralin absorption spectrum since literature data are rather scarce [3, 5].

The absorption spectra of anthralin, 1,8-dihydroxyanthraquinone and 1,8-1′,8′-tetrahydroxydianthron in nonaqueous media are given in Fig 1. In aqueous solutions the presence of ionizable hydroxyl groups on the anthracene ring, leads to the occurrence of an ionic equilibrium between the neutral and the monobasic form of anthralin as evidenced on Fig 2*A* which shows an isobestic point (and thus the presence of only 2 species at equilibrium) at 360 nm. The pKa can be determined accurately enough from Fig 2*B* data although the low solubility of anthralin at neutrality and its unstability at pH > 12 imposes the spectral analysis in the pH range 7–12. This pKa is found to be 9.4 in complete disagreement with previous determination [11] which in 75% dioxane–25% water mixtures led to pKa = 13.06. However, the entirely different medium conditions do not allow direct comparison between these 2 values. It turns out that the 1,8-dihydroxyanthraquinone pKa is also found to be 9.4. We have no explanation at hand for this similarity.

In conclusion, since the poor solubility of the dianthron (III) precludes any valuable pKa determination, these ionic equilibria can be depicted as follows.




It is interesting to note that in contrast with the optical absorption spectra recorded in chloroform or acetone, the one recorded in ethanol shows a shoulder at ~ 392 nm indicating, in neat ethanol, a low concentration of the basic form of anthralin (Fig 1). The stabilization of ionic species in a nonionizing solvent like ethanol is not unusual since it occurs for instance with quinoline derivatives [12]. It has been attributed to strong hydrogen bonding of solvent molecules with weakly acidic groups. In aprotic solvents like the basic dimethylformamide (DMF) [5], the absorption spectrum of the anionic anthralin is obtained (Fig 2) while in DMSO both the neutral and anionic form are present (Fig 2).

### *Fluorescence Studies on Anthralin and Oxidation Products*

Neutral aqueous anthralin solutions do not fluoresce. Increasing the pH leads to the appearance of a fluorescence emission the excitation spectrum of which clearly corresponds to the absorption of the anthralin monoanion. Upon excitation at 450 nm which selectively excites the monoanion, the fluorescence quantum yield reaches 0.1 at pH 9.4 as measured using the fluorescein emission as standard [13]. In DMF, as expected in view of the absorption properties in this solvent, the same fluorescence emission is recorded (Fig 3) but the fluorescence quantum yield is dramatically increased ($\phi_F = 0.7$). the fluorescence quenching observed in basic aqueous medium as compared with the same emission in DMF is due to strong interactions between anthralin monoanions in their first excited

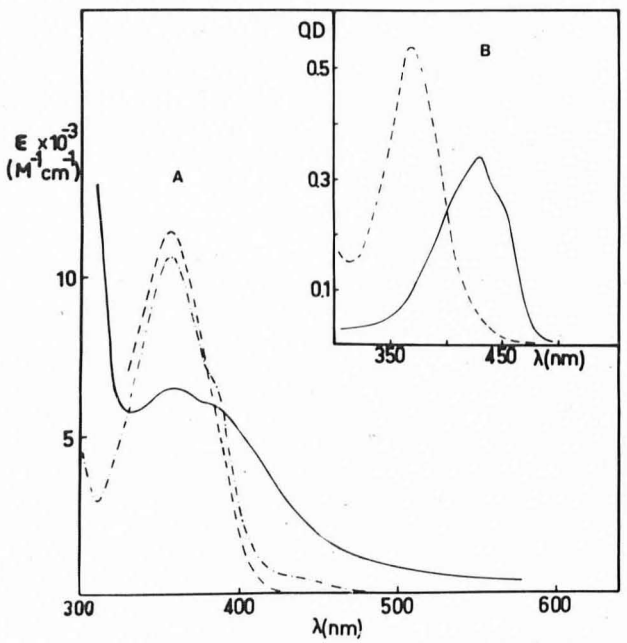



FIG 1. *A*, Absorption spectrum of anthralin (20°C) in (———) aqueous solution (pH 7.5) (– – –) acetone or chloroform (–·–) ethanol. *B*, Absorption spectrum of 1,8-dihydroxyanthraquinone (———) and 1,8-1′,8′-tetrahydroxydianthron (– – –) in ethanol (20°C).

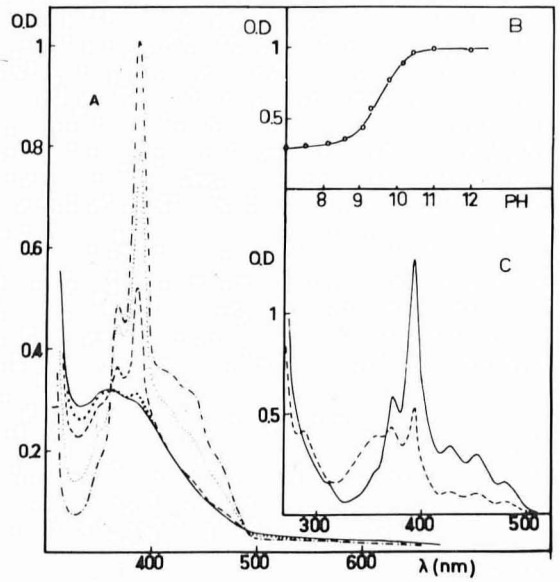



FIG 2. *A*, Absorption spectrum (20°C) of aqueous anthralin at: (———) pH 7.55; (×××) pH 8.10; (– – –) pH 9.00; (. . .) pH 9.8; (–·–·–) pH 11.0. *B*, Titration curve of $5 \times 10^{-5}$ M anthralin in $5 \times 10^{-2}$ M Tris buffer determined from absorbance at 384 nm ($\epsilon$ = 18,000 $M^{-1}$ cm for the anthralin anion). *C*, Absorption spectrum (20°C) of $5 \times 10^{-5}$ M anthralin in (———) DMF; (– – –) DMSO.

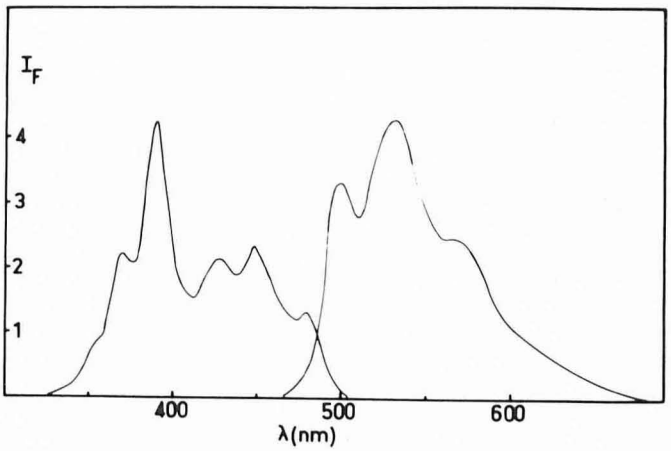



FIG 3. Corrected fluorescence excitation (emission set at 500 nm) and emission (upon excitation at 450 nm) spectra of anthralin in DMF. The emission intensity is 70 times lower in acetone which leads to the same spectrum. Temperature 4°C.

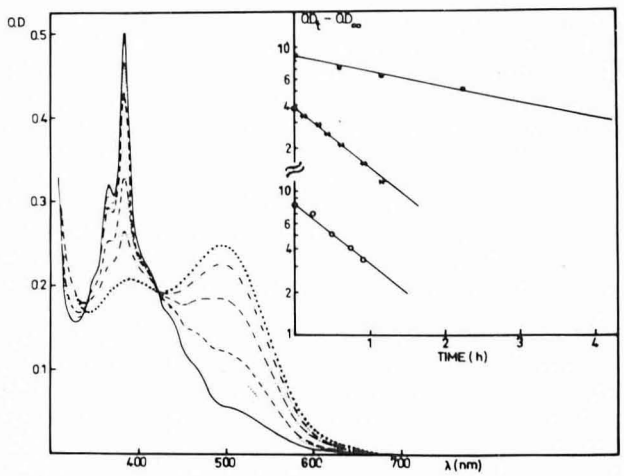



FIG 4. Anthralin autooxidation in buffered solutions at pH 10.4; $[O_2] = 2.65 \times 10^{-4}$ M. Solutions were kept in the dark: temperature 20°C. *Inset*: Kinetics of anthralin oxidation in ethanol (□—□), pH 10.4 buffer (*–*), dimethylformamide (○—○) measured at 388 nm. Anthralin, concentration: $2.5 \times 10^{-5}$ M.

singlet state (from which the fluorescence emission arises) and the strongly polar water molecules, leading to nonradiative deactivation as shown by studies with other aromatic molecules such as indole derivatives [14].

A weak anthralin monoanion fluorescence emission is observed in ethanol and an even weaker one in acetone, confirming the optical absorption properties and the presence of monoanionic anthralin in these hydrogen bond-forming solvents. The excitation spectrum of this fluorescence emission corresponds to the absorption spectrum of the anionic species ruling out that an excited state proton transfer is responsible for the fluorescence emission [15] since in this case the fluorrescence excitation spectrum would correspond to the absorption spectrum of the neutral species.

The oxidation products II and III do not fluoresce in the present experimental conditions.

### *Effect of Solvent, pH, Light and Complex Formation on the Stability of Anthralin*

There are some reports on the stability of anthralin. However they suffer from a major flaw, i.e., their qualitative aspects. For instance the solvent and light effects are not satisfactorily separated leading to somewhat confusing data [5,16] on the anthralin instability. This oxidation has been shown to involve free radicals and possibly $O_2$-radicals [17].

*Effect of solvent and pH:* Although anthralin is fairly stable in chloroform, it is slowly oxidized in alcohol, acetone or in neutral aqueous solutions where the oxidation rate increases with increasing pH. In dimethylformamide and basic solutions the oxidation is very fast, leading in the primary step to the dihydroxyanthraquinone (II) which is fairly stable in basic media where it slowly decomposes into further products, among them compound III and other unknown compounds related to the so-called anthralin brown [4]. The kinetics of this oxidation reaction is first order (Fig 4) at low anthralin concentration which can be simply explained by the following scheme.

$$I^- + O_2 \xrightarrow{k_1} \text{Products} \tag{1}$$

$$\text{leading to } -\frac{d[I^-]}{dt} = k_1[O_2][I^-] \tag{2}$$

In our experimental conditions $[I^-] \simeq 1\text{–}3 \times 10^{-5}$ M and under air saturation, $[O_2] \simeq 10[I^-]$ so that (2) can be represented as a pseudo first order reaction characterized by a lifetime $\tau = 1/k_1[O_2]$.

In the Table the reaction rate constant for the anthralin oxidation in various solvents is given.

From data in the table it can be concluded that the autooxidation of the monobasic species is much more rapid than the neutral one and is rate determining in mixtures of both species at equilibrium. It can thus be suggested that in alcoholic solutions the autooxidation still proceeds via the monoanionic species formed at low concentration as shown by fluorescence spectroscopy. The general scheme for the autooxidation of anthralin could be described as:

$$I \overset{K}{\rightleftharpoons} I^- + O_2 \xrightarrow{k_1} \text{Products} \tag{3}$$

K is the stability constant of the $I^-$ species in the nonaqueous medium and $k_1$ is the bimolecular reaction rate constant of (eq. 1):

$$-\frac{d[I^-]}{dt} = k_1[I^-][O_2] \tag{4}$$

$$-K\frac{d[I]}{dt} = Kk_1[I][O_2] \tag{5}$$

$$-\frac{d[I]}{dt} = k_1[I][O_2] \tag{6}$$

The reaction rate constant for the autooxidation of anthralin in ethanol is given in the Table.

*Effect of light:* In contrast with the autooxidation in the dark both the neutral and the monoanionic species are readily oxidized (Fig 4) by UV or visible light. As shown on Fig 5 in ethanolic solutions, the presence of good isosbestic points suggests that there is a single photooxidatin product, namely the dihydroxyanthraquinone. The same considerations apply to neutral or basic aqueous solutions although in basic solution the photooxidation leads to the formation of the monobasic form of the dihydroxyanthraquinone (II). The photooxidation quantum yields (i.e. the number of anthralin molecules oxidized per absorbed quantum) under various experimental conditions are given in the Table. They are reasonably high with respect

TABLE *Characteristics of anthralin oxidation*

| Solvent | DMF, $H_2O$ (pH 10.4) or $H_2O + 1.4 \times 10^{-1}$M HSA | Ethanol |
|---|---|---|
| $k_1$ | 0.5 $M^{-1}sec^{-1}$ | 0.05 $M^{-1}sec^{-1}$ |
| $\phi$ | $5 \times 10^{-3}$<br>Irradiation wavelengths:<br>404 and 437 nm | $4 \times 10^{-3}$<br>Irradiation wavelengths:<br>>320 nm |

Second order rate constant ($k_1$) for the autooxidation and quantum yield ($\phi$) for the photooxidation of anthralin in various conditions at 20°C. $[O_2] = 2.65 \times 10^{-4}$ M in an air-saturated $H_2O$ and $2.07 \times 10^{-3}$ M in air-saturated ethanol.

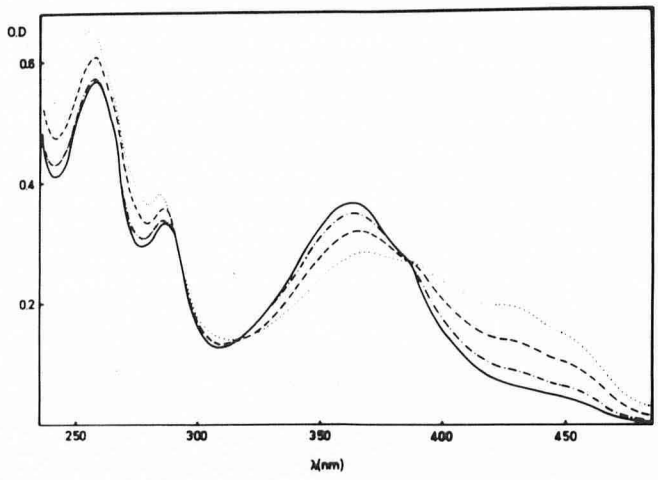



FIG 5. Photodegradation of $3.5 \times 10^{-5}$ M anthralin in ethanol: $I_{abs} = 4.9 \times 10^{-8}$ einstein $sec^{-1}$. Temperature 20°C. $[O_2] = 2.07 \times 10^{-3}$ M (air saturated ethanol).

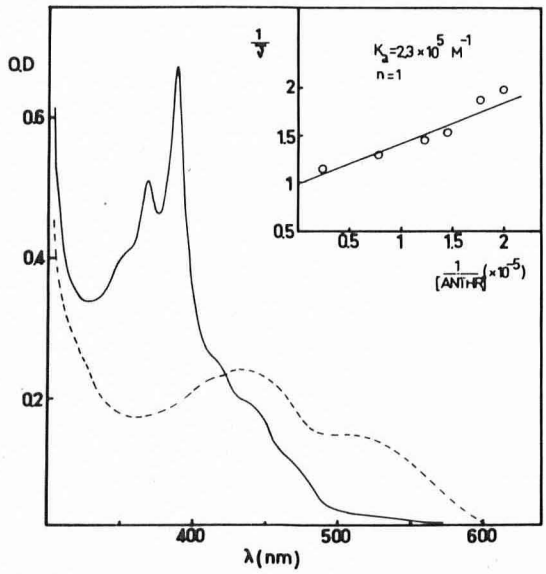



FIG 6. Absorption spectra (20°C) of anthralin (———) and 1,8-dihydroxyanthraquinone (- - -) bound to HSA in pH 7.4 Tris buffer. *Inset*: Benessi-Hildebrand reciprocal binding plot for the anthralin HSA complex.

to most common photooxidation encountered in biological systems [18].

As shown on Fig 5, the photooxidation is a first order process, which must be expected in view of the short lifetime of the excited states of organic molecules produced by the excitation with visible light. It is also consistent with the small molar extinction coefficient and the weak light intensity used in steady state irradiations [13].

*Spectroscopic Investigation of the Interaction of Anthralin and Derivatives with Albumin and DNA*

*Albumin:* Addition of HSA or BSA to a buffered neutral aqueous anthralin solution leads to a bathochromic shift of the anthralin absorption and the appearance of the characteristic absorption of the monobasic species (Fig 6) indicating complex formation between albumin and anthralin. Similarly the 1,8-dihydroxyanthraquinone (II) binds to HSA leading to the monoanionic species. On the other hand, the dimeric species (compound III) does not form complexes with HSA.

*DNA:* The slow decomposition of the neutral species in neutral aqueous solutions (see above) has some important consequences. The apparent shift of the anthralin absorption spectrum in presence of DNA in neutral aqueous solutions occurring after incubation times up to 20 hr [19,20] is not necessarily due to binding of anthralin to DNA but rather to the slow oxidation of anthralin. Thus, no optical absorption red shift can be detected at either room (20°C) or high temperature (65°C) (where DNA is either native or denatured) when experiments are peformed with deaerated mixtures. In addition, in deaerated solutions, there is no apparent increase in the solubility of anthralin at neutral pH in presence of 0.1% DNA.

*Stability of the Albumin-Anthralin Complex:* Since the binding of anthralin to HSA results in the formation of the monobasic anthralin, anthralin is readily oxidized (see Table, Figs 4–6) either in the dark or upon excitation with UV and/or visible light yielding again the 1,8-dihydroxyanthraquinone (compound II) which binds to HSA where it is stabilized as the monoanion. This latter species slowly oxidizes leading to brown products.

*Spectroscopic Investigation of Anthralin Stability in Human Epidermis*

It has been shown in the preceding section, that anthralin binds to proteins such as serum albumins. It can thus be anticipated that binding to proteins occurs when anthralin is applied on human skin. The absorption spectra of intact human epidermis samples (Fig 7) treated with anthralin show the presence of strong absorptions in the 400–600 nm region indicating the formation of oxidation products. Their nature depends on the time elapsed after the topical application and not on the mode of application (i.e., 0.5% anthralin in cream or in acetone). The shape of the absorption spectrum suggests the formation of the dihydroxyanthraquinone as the primary product since it gives rise to an absorption maximum in the 500 nm region when bound to protein.

The strong absorption band in the 380 nm region strongly suggests that unoxidized anthralin is still present in human skin 24 hr after topical application. In support to this hypothesis *in vitro* experiments show that incubating epidermis with $5 \times 10^{-5}$ M anthralin (prepared as a 2% stock solution in acetone) in Hanks' medium leads to an absorption spectrum (and thus to products) not essentially different, after 3 hr (except in the relative proportion of oxidized versus unoxidized anthralin), from those obtained with *in vivo* topical application (Fig 7). Moreover unoxidized anthralin can be still clearly identified in human skin 24 hr after incubation by its fluorescence emission. As shown on Fig 8 where, besides an unidentified fluorescence

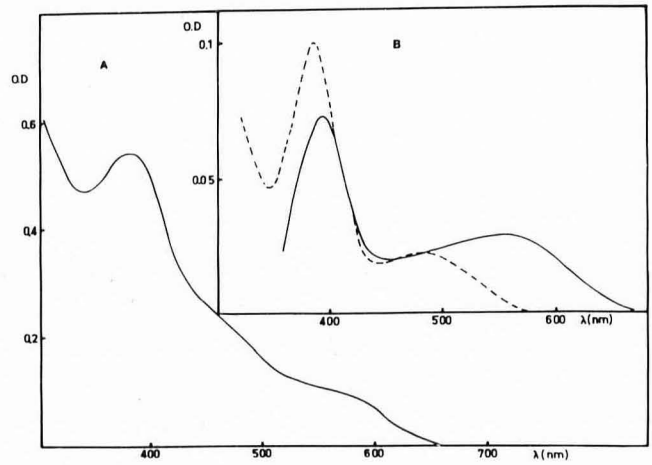



FIG 7. *A*, Absorption spectrum of a normal skin epidermis incubated for 3 hr with $10^{-4}$ M anthralin in Hanks' medium. *B*, Absorption spectra of a nonpsoriatic patient skin epidermis treated with 0.5% anthralin cream or acetone solution. (———) Treatment before blister formation, (- - -) treatment after blister formation. Temperature in all cases: 20°C.

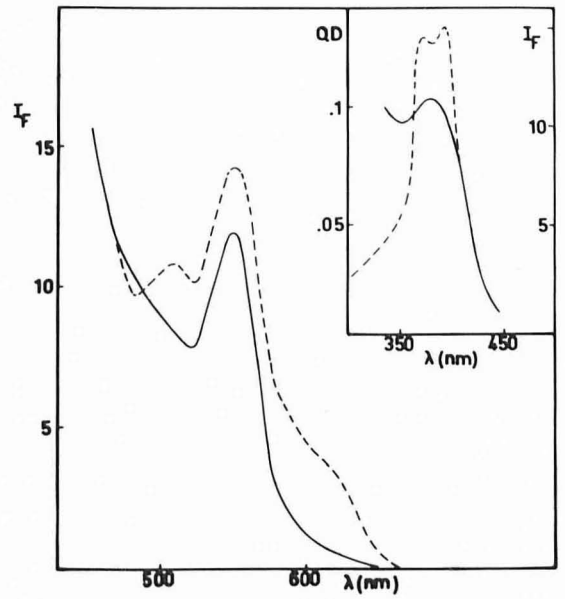



FIG 8. Corrected fluorescence spectra (excitation wavelength: 390 nm) of an incubated human epidermis sample. (———) Without anthralin, (- - -) with anthralin ($2 \times 10^{-4}$ M in Hanks' medium). *Inset*: Absorption (———) and fluorescence (- - -) excitation spectra; the fluorescence excitation spectrum was recorded with the emission monochromator set at 550 nm.

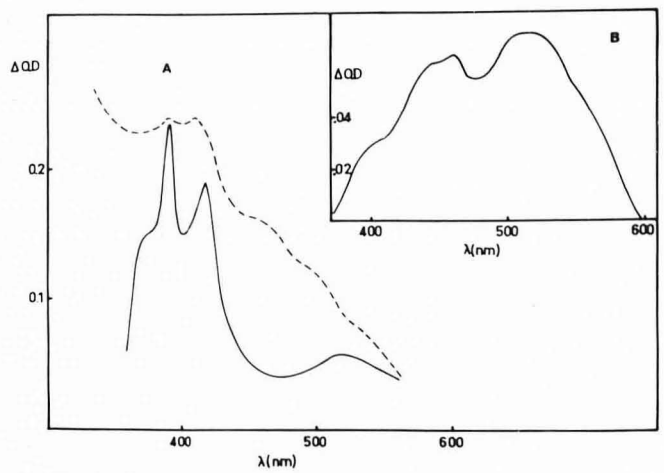



FIG 9. Absorption spectra of suction blister fluid (measured at 20°C). *A*, Doped with $5 \times 10^{-5}$ M anthralin; ——— immediately after addition; - - - 4 days after addition. *B*, Doped with $5 \times 10^{-5}$ M, 1,8-dihydroxyanthraquinone.

emission, the presence of the characteristic fluorescence maximums at 510, 550, and 600 nm and the fluorescence excitation spectrum corresponding to the absorption spectrum well characterize the anthralin monoanion.

### *The GCMS Detection of Anthralin and 1,8-Dihydroxyanthraquinone in Suction Blister Fluid*

Anthralin and dihydroxyanthraquinone can be easily identified in suction blister fluid doped with known amounts of these derivatives (Fig 9). Interestingly enough, absorption spectroscopy shows that, in this case, both compounds bind to albumin which constitutes an important component of the interstitial fluid [9]. Such a binding leads, of course, to a fast oxidation of anthralin as observed in model buffered albumin solutions because of the anthralin monoanion formation and its easy oxidation.

Such solutions also represent excellent standards for a fragmentation study and characterization by GCMS. As already reported [10] anthralin and 1,8-dihydroxyanthraquinone but not the "anthralin dimer" (III) can be identified by mass spectrometry provided they are stabilized as trimethylsilyl derivatives after chromatography (see Experimental section). In these conditions, the retention times are 6.9 mn and 7.9 mn for tri-TMS-anthralin and bis-TMS-1, 8-dihydroxyanthraquinone respectively (see Experimental). Their mass spectrum (Fig 10) reveals characteristic ions at m/e = 442 (anthralin) and m/e = 369 (1,8-dihydroxyanthraquinone) allowing their determination and quantification using the single ion monitoring technique (SIM). The lowest concentration that can be detected using this technique is $0.04 \times 10^{-10}$ M [10].

SIM centered on m/e = 442 and 369 and carried out on silylated derivatives makes it possible to detect without, of course, any difficulties $10^{-5}$ M anthralin and 1,8-dihydroxyanthraquinone in 500 $\mu$l of doped suction blister fluid (Fig 11). On the other hand, the same determination carried out on suction

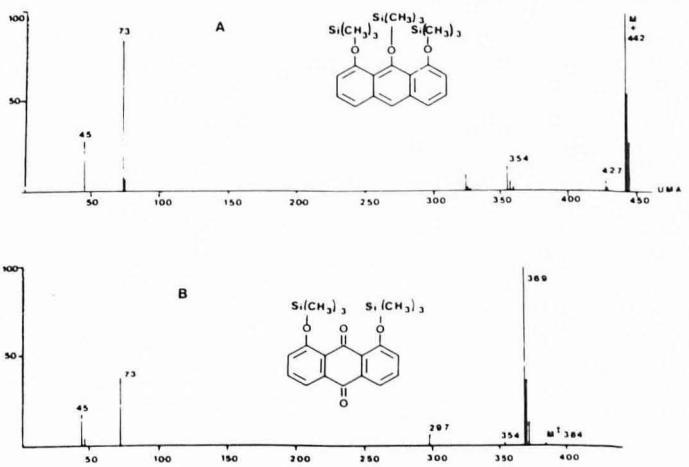



FIG 10. *A*, Mass spectrum of silylized anthralin. *B*, Mass spectrum of silylized 1,8-dihydroxyanthraquinone.

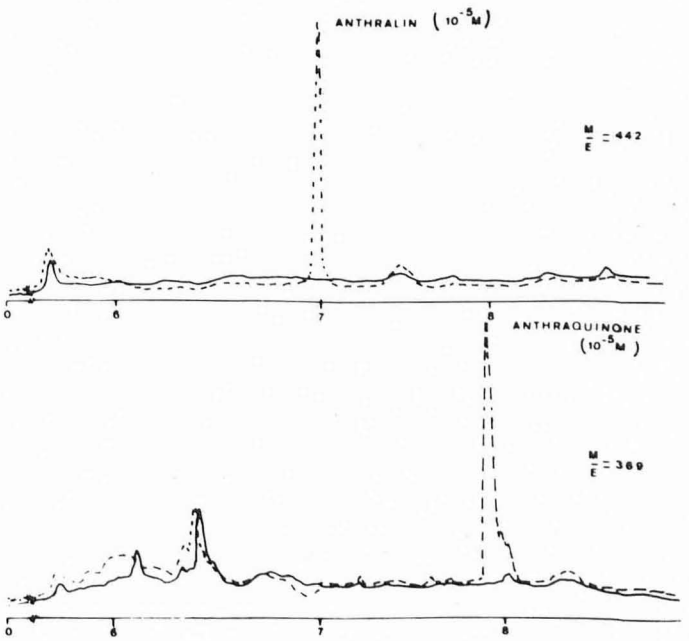



FIG 11. Single ion monitoring on suction blister fluid: - - - Doped blister fluid; ——— blister fluid after topical application of anthralin on intact human skin.

blister fluids obtained from suction blisters done on intact human skin topically treated with anthralin does not provide any evidence for these derivatives.

## DISCUSSION

For the sake of clarity the basic physicochemical properties of anthralin have been discussed along with the presentation of the results. Because of its obvious biological implications, the complex formation with human serum albumin deserves much attention. This complexation with HSA was studied according to a Scatchard analysis [21]:

$$\frac{\bar{v}}{C} = K(n - \bar{v}) \quad (7)$$

where $\bar{v}$ is the concentration ratio between bound anthralin and the protein, C is the concentration of unbound anthralin and n the average number of bound anthralin molecules per protein molecule. The molar extinction coefficient and thus the concentration of bound anthralin was obtained from the fraction of light absorbed at 398 nm at saturation of the macromolecule by the anthralin ligand.

A Benessi-Hildebrand reciprocal binding plot is presented in inset of Fig 6 leading to $K = 2.3 \times 10^5\ M^{-1}$ with one binding site. It must be noted that at high (anthralin)/(HSA) molar ratios the same analysis shows that anthralin can occupy on the average 2.5 sites. A similar association constant was found for the 1,8-dihydroxyanthraquinone. This result, with the fact that the monoanion is formed upon complexation, suggests that these 2 molecules bind to the same site on HSA. This indicates that the binding site has a high proton affinity corresponding to a local pH shift of about 2 pH units with respect to the buffered aqueous solutions.

Thus, provided anthralin passes through the epidermis during an acute inflammatory response, its fast binding to HSA contained in the interstitial fluid [9] induces an almost instantaneous oxidation to dihydroxyanthraquinone which thus could be believed as directing the metabolic path, leading to the anthralin degradation products found in biological fluids. On the other hand, the spectral shift observed in aerated mixtures of anthralin and DNA is clearly shown not to be a proof of DNA-anthralin complex formation [19, 20]. Such studies require anaerobic conditions and more elaborated and sensitive techniques than optical absorption measurements. In addition, it must be noted that quinacrine (which binds to DNA) and anthralin inhibit UV-induced DNA repair at different steps by different mechanisms [22].

The presence of the monoanionic species in human skin as clearly shown by fluorescence spectroscopy reveals that at least some anthralin binds to skin proteins at sites whose structures are close to those found in albumin. One may argue it is somewhat surprising that anthralin, which is fairly unstable in aerated solutions especially in the monoanionic form, is much more stable in air-exposed human epidermis. In fact, it is well established that oxygen diffusion in proteins does not follow the simple diffusion kinetics observed in solutions so that molecules that are buried in protein structure are not fully accessible to oxygen. Liver alcohol dehydrogenase [23] or chymotrypsin [24] are good examples of such properties since the phosphorescence of a tryptophan residue of the protein or the phosphorescence of an extrinsic chromophore (benzophenone) bound to the protein can be detected at room temperature in aerated solution, although the characteristic property of a phosphorescence emission is to be quenched by oxygen.

It must be noted that the absence of anthralin in suction blister after topical application is in agreement with a strong binding of anthralin to skin structures impeding important diffusion towards dermis through the interstitial fluid. This is also in agreement with previous studies [25] showing that most of the ointment and its ingredients remains at the surface of the skin although the nature of the ointment does influence the penetration into the epidermis [25]. However it must be stressed that our negative result on the anthralin content of the blister fluid has been obtained after topical application on healthy skin. Such a study should be extended to psoriatic patients showing skin lesions.

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