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Wound healing properties of naphthaquinone pigments from *Alkanna tinctoria*

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Summary. From the roots of *Alkanna tinctoria* Tausch, the alkannin esters of the following acids were isolated and identified: β,β-dimethylacrylic acid, β-acetoxy-isovaleric acid, isovaleric acid, and angelic acid. These esteric pigments showed excellent wound healing properties in a clinical study on 72 patients with ulcus cruris.

The healing of a wound, although among the oldest of surgical problems, retains many of its secrets. However, some wounds become contaminated or contain foreign material that cannot be removed during the acute inflammatory reaction. A condition of chronic or atonic ulcer then appears, for whose treatment there is a lack of effective drugs. Conventional treatment usually entails the prevention of infection and any further aggravation of the wound. If a risk of septicemia exists, amputation may be advised.

As stated in the 'Greek Herbal of Dioscorides'[2] compiled in the 1st century, the roots of the plant *Anchusa tinctoria* or *Alkanna tinctoria*[4] were used for the healing of wounds: 'The root is of a binding nature, being good for burnings and old ulcer...'. But since that time, the medicinal value of this plant has either been forgotten or assumed to be nothing but folklore. This paper describes the rediscovery and verification of this medicinal property, as well as the determination of the active components of the root.

A series of trials with different extracts of the roots of *Alkanna tinctoria,* on skin ulcers experimentally induced on lab animals (rats, cats, dogs), indicated an excellent healing effect[4]. Subsequent to these findings, studies were carried out to determine the active principles in these extracts and also to formulate a suitable pharmaceutical preparation for clinical studies. For this purpose, a large number of root samples of *Alkanna tinctoria,* obtained from different locations, were phytochemically analyzed[5]. The initial hexane extract, after further extraction and chromatographic separation, was divided into 4 fractions: waxes[6], fluorescent compounds, natural polymers[5] and pigments[7]. Of these fractions, only that containing the pigments showed a healing effect when tested experimentally on skin ulcers. Following these preliminary results, the investigation was focused on the pigment-containing fractions, from which I isolated and identified the following esters of alkannin (**1a**): β,β-dimethyl acrylate, **1b**[5], angelate, **1c**[8], isovalerate, **1d**[8], and the novel β-acetoxy isovalerate, **1e**[9]. Brockmann has reported that the structure of the pigment found in *Alkanna tinctoria* was the levorotatory form of 5,8-dihydroxy-2-(1-hydroxy-4-methylpent-3-enyl)-1.4-naphthaquinone (alkannin), **1a**[10]. Contrary to Brockmann's findings, however, I was unable to isolate free alkannin from any of the samples analyzed, irrespective of their origin. I believe that Brockmann's alkannin was an artifact, caused by the caustic alkali which he used for the isolation of the pigments.

Following the chemical and animal studies described above, a pharmaceutical ointment was formulated[11]. Since this ointment is to be used for topical application to human skin, it was necessary first to test it, as well as its active ingredients, for mutagenicity in Ame's Test[12]. The ointment

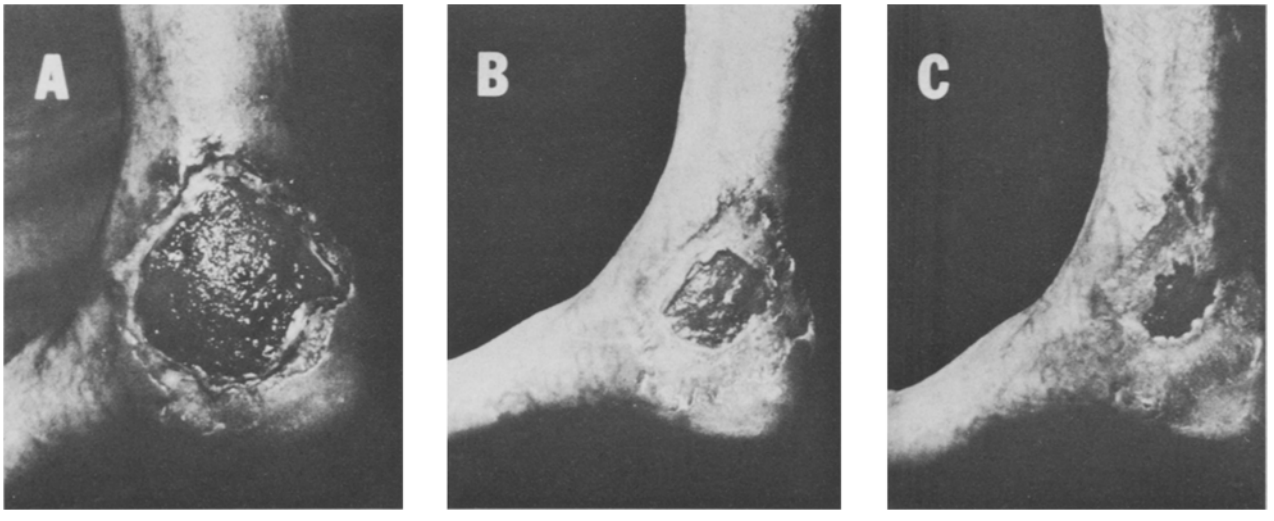


Fig. 1. Showing the response to alkannin esters of a severe case of ulcus cruris: *A* before treatment, *B* after 2 weeks, and *C* after 18 weeks of treatment.

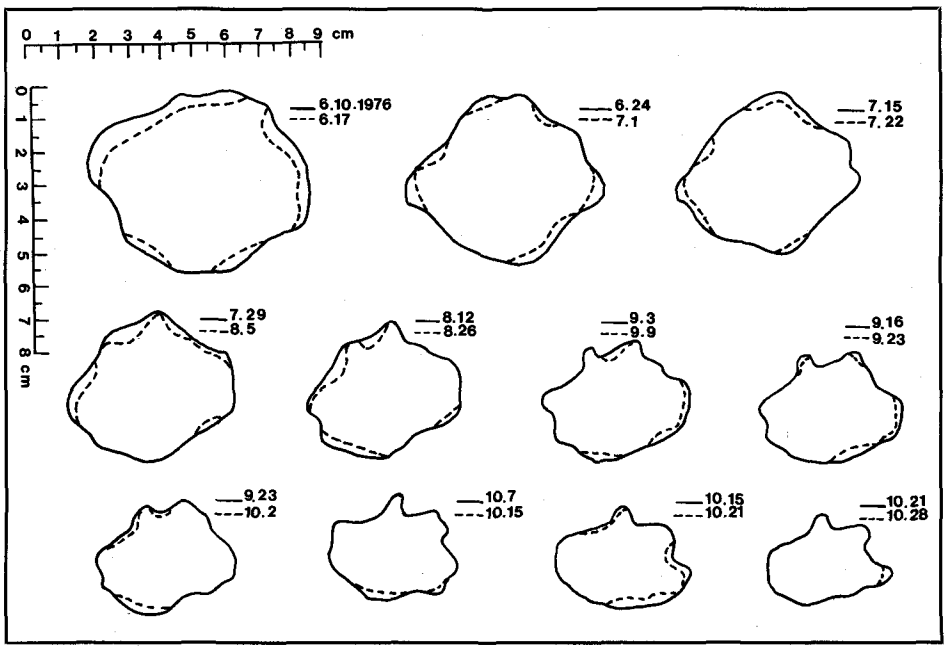
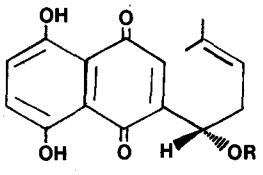


Fig. 2. Diagrammatical presentation of the cicatrization of the ulcer of the same patient during the period of treatment (6.10.76 to 10.28.76) at the dermatological ward of the Heidberg Hospital (Hamburg, Germany).



1

a. R = H

b. R = $-COCH=C(CH_3)_2$

c. R = $-COC(CH_3)=CHCH_3$

d. R = $-COCH_2CH(CH_3)_2$

e. R = $-COCH_2C(CH_3)_2-OCOCH_3$

f. R = $-COCH_3$

and the alkannin esters at noninhibitory concentrations, in presence or absence of Aroclor-induced rat liver enzyme, did not increase the frequency of mutations in TA 98 and TA 1535 strains of *Salmonella typhimurium*. The active ingredients, however, inhibited the growth of *Salmonella* strains at 25 µg/plate or higher concentrations. The ointment was then tested for the inhibition of microbial growth. After application onto *Petri dishes*, the bactericidal action of the active ingredients of the ointment was demonstrated[13]. Since the active ingredients are not water-soluble, the method of growth index on contact, according to F. Heiss, was used[14].

Subsequently, clinical studies were undertaken over a period of 3 years, on 72 patients suffering from ulcus cruris (indolent ulcer) on the lower part of the leg, due to varicose veins. They had all been previously treated with various commercially available drugs indicated in the treatment of ulcers, but without success; the ulcer resisted treatment either because of toughening of the connective tissue around it, or because of inadequate blood perfusion. After thorough cleansing of the ulcer, the ointment was applied daily as a thin film on a radius 5–10 cm around the ulcer. The dressings were changed daily and the therapeutic effect of the ointment was evaluated. During the 1st or 2nd week of treatment, the base of the ulcer cleared with concurrent degradation of the necrotic tissue. During the subsequent 2 weeks, proliferous growth of granulation tissue, as well as epithelization of the edges of the ulcer, were observed. Subsequently, more clearing as well as softening of the ulcer was observed. Generally, a treatment of 5–6 weeks resulted in complete healing, or in considerable reduction in the size of the ulcer. Furthermore, no skin inflammation was observed during therapy. The percentage of success was 80%. The dramatic improvement in a particularly severe case of ulcus cruris is shown in figure 1. Figure 2 shows the fate of the ulcer diagrammatically, during the same period[16].

It is noteworthy that the alkannin esters **1b** and **1f** have been demonstrated to be active also against Walker carcinosarcoma in rats[17]. However, wound-healing properties are not normally associated with anticancer activity, since several potent antitumor agents such as adriamycin, actinomycin D, and 5-fluorouracil do not display wound-healing properties[18]. The esters of alkannin described in this paper display a remarkable ability to regenerate necrotic tissue and may well prove to be of considerable therapeutic value.

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Cerebral edema in the rat with galactosamine induced severe hepatitis

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Summary. With D-galactosamine hydrochloride severe hepatitis was induced in rats and the water content of cerebrum, cerebellum and brain stem determined. The animals showed a parallel increase in cerebral water content and occurrence of cerebral symptoms.

Patients with endogenous liver coma (acute liver necrosis) may show symptoms that are characteristic for compression of midbrain (such as large pupils, no light reaction of pupils, decerebration rigidity, tachypnea, hyperthermia) and of medulla oblongata (such as apnea, sudden drop in blood pressure, areflexia). None of these symptoms is indicative of elevated pressure of the brain since these signs could also be of metabolic origin[1]. Several authors have found cerebral edema at autopsies on patients who died of endogenous liver coma (table 1)[1-6]. In 1971, Ware et al. observed the presence of cerebral edema in 16 out of 32 patients, 4 of whom exhibited herniation of the cerebellar tonsils or uncal herniation[4]. In 1972, Thölen reported on 16 patients all with cerebral edema, 9 with impression of the tentorium cerebelli and 7 with hernia of the tonsilla cerebelli[1]. Williams et al. found cerebral edema in 13 out of 16 patients[5]. Subsequently, the same authors studied the autopsy reports and discovered cerebral edema in 40 out of 105 cases with massive liver necrosis[6]. Therefore it may be concluded that, in cases of endogenous liver coma, the cause of death is often cerebral edema. Clinically, the question may be raised whether in acute liver necrosis all symptoms of the coma are due to cerebral edema. Further it is of interest to know whether early prophylaxis and treatment of cerebral edema would give the liver cells sufficient time to regenerate.

In the present study, the water content of different parts of the brain (cerebral hemispheres, cerebellum, brain stem) was determined in rats with severe acute hepatitis induced by D-galactosamine hydrochloride. The following questions were investigated: Does cerebral edema occur when liver failure is induced experimentally? Can this be evaluated quantitatively? When and in which part of the brain does it occur? With what symptoms can it be correlated?

Methods. Female albino rats with a weight of 180–200 g were given i.p. 2.5 g D-galactosamine hydrochloride/kg b. wt (solution: 0.45 N, pH value at 7.4 with 1 N NaOH). During the course of the experiment, food and water were not limited. The animals were killed by decapitation 24, 36, 42, 48, 54 h after the injection, and in another experiment the animals were not killed until the onset of severe cerebral symptoms independent of the time of galactosamine injection. The brain was immediately removed and dissected into the different parts: the 2 cerebral hemispheres, cerebellum and brain stem. The water content of the above-mentioned parts was determined by calculating the difference between the wet weight and the dry weight. The specimens were dried in an oven at 100 °C for 24 h. 24 h after the galactosamine injection, a 4-ml blood sample was taken from 10 rats by heart puncture (4 ml blood + 1 ml sodium citrate 3.8%) and the plasma used to determine the prothrombin time (reagent used: activated rabbit brain thromboplastin, DADE).

Results. The average values for the water content of the different cerebral regions removed from untreated control animals are given in table 2. Animals treated with galactosamine:

- A dose of 2.5 g/kg D-galactosamine hydrochloride is lethal for albino rats with a weight of 180–200 g. Preliminary experiments have shown that most of the animals die 40–56 h after the injection.
- From the 36th h on, the animals become increasingly sleepy and inactive.
- Prior to death, the animals passed through phases of various duration of total immobility (no reaction at all to optical, acustic or tactile stimuli). Also violent trembling and hyperactive phases with excessive and uncontrolled motor activity were observed (frequent and sudden jumps with transition to severe convulsive spasms).
- 24 h after the injection, most animals showed an abnormal tendency to bleed.
- 24 h after the injection, the Quick value for all animals investigated was less than 3% (average prothrombin times for untreated animals = 100%).
- The water content of all cerebral regions remained more or less constant over a 24-h period. Only the cerebellum showed a tendency toward water retention 24 h after the injection. 36 h after administration of galactosamine, the water content of the cerebellum was significantly higher, i.e. by 0.45% when compared to the control values (t-test according to Student: $p < 0.01$), after 42 h by 0.77% ($p < 0.001$). For this time period, a significant increase in the water content of the brain stem was also noted: 0.54% ($p < 0.05$). 48 and 54 h after the galactosamine injection, the water content of the cerebellum remained significantly high, whereas cerebral hemispheres and brain stem began to dry out.

The largest water increase in the brain stem was observed in rats which were killed when extremely severe cerebral symptoms were evident (as a rule 43–56 h after injection).

Table 1. Autopsies of patients died in endogenous liver coma

Author	Autopsies	Cerebral edema (number)	Cerebral edema (%)
Ware[4]	32	16	50
Thölen[1]	16	16	100
Williams et al.[5]	16	13	81
Hanid et al.[6]	105	40	38