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[54] **C22 ACID AND ITS SALTS TO PROMOTE WOUND HEALING**

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[51] **Int. Cl.** **C08h 17/36**

[58] **Field of Search** 260/413; 424/344, 318

[56] **References Cited**

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[57] **ABSTRACT**

A C22 homolog of retinoic acid and its salts have been found effective in promoting would healing. The acid or the salt is applied to the wound as a solution, ointment or powder.

2 Claims, No Drawings

C22 ACID AND ITS SALTS TO PROMOTE WOUND HEALING

SUMMARY OF THE INVENTION

Inflammation and mucopolysaccharide synthesis are the two important features in the early stage of wound healing. The term "wound" as used in this application means any topical lesion such as a surgical incision, accidental wound or ulcer. Aspirin inhibits both features. The healing inhibitory action of aspirin and other inflammatory agents has been demonstrated. Vitamin A increases mucopolysaccharide synthesis and it also causes inflammation. The ability of vitamin A alone to promote healing and its effectiveness in reversing the healing retardation action of aspirin is known. Retinoic acid (the acid form of vitamin A) and its salts also have been found active compounds in promoting healing. Topical application of retinoic acid or its salts reverses the healing retardation action caused by oral administration of sodium salicylate, prednisone and other inflammatory agents and topical application of salicylic acid or hydrocortisone. Topical application of retinoic acid and its salts promotes skin wound healing in rats and human beings.

It has now been found that 2,6,6,-Trimethyl-1-(10'-carboxy-3',7'-dimethyl-deca-1',3',5',7',9',-pentaenyl) cyclohex-I-ene or C_{22} Polyene Acid and its salts is even more effective than retinoic acid for this purpose. Further the C24 homolog has been tested and found less effective than the C22 acid of the present invention. The acid of the present invention is sometimes referred to as C22 acid for convenience.

Clinically, hydrocortisone, prednisone and salicylic acid are very commonly used. Hydrocortisone and salicylic acid preparations for topic uses are quite popular. Whitfield Ointment, USP, contains 3% salicylic acid. Prednisone is a potent synthetic analogue of cortisone which is only used orally. It has been used in a large variety of diseases and it is not uncommonly used on surgical patients to reduce edema or inflammation. Corticosteroids are used in organ-transplant surgery to suppress immunological response. Sodium salicylate has been employed in the symptomatic therapy of acute rheumatic fever for many decades. Oftentimes it is still the drug of choice in many incidences. However the salicylates, hydrocortisone, prednisone, indomethacin, mefenamic acid, retard healing. The retardation action of these drugs can be reversed by applying C22 acid or its salts on the wound. These findings illustrate the principle that one can use a second drug (C22 acid) to modify the untoward effect (wound healing retardation) of a useful drug (anti-inflammatory agents).

C22 acid or its salts alone promotes healing. It is very practical to dust this compound on any surgical wound or to apply it as a solution or in an ointment.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The local application of C22 acid and its salts has been found to promote wound healing. This is true both of animals which have not been otherwise treated and also true of animals which have been treated with antiinflammatory agents such as a salicylate, hydrocortisone, prednisone, indomethacin, mefenamic acid and the like. These compounds normally retard healing and

C22 acid and its salts has been found to reverse this action.

The C22 acid, namely 2,6,6-trimethyl-1-(10'-carboxy-3',7'-dimethyldeca-1',3',5',7',9',-pentaenyl)-cyclohex-1-ene acid is a novel compound and can be made as follows:

Sodium hydride (57% in mineral oil) was washed with anhydrous ether. To a suspension of this NaH in anhydrous ether, triethylphosphonoacetate was added with stirring. To the clear solution, retinal was added at 0° C over 15 min. The reaction mixture was kept at 0° C for 30 minutes, and then at 35° C for an additional 30 minutes. Saturated aqueous sodium chloride was added to the mixture and cooled to 0° C. Extraction with petroleum ether (30°–60°), drying and removal of solvent gave a dark red oil.

Because of the suspected lability of this ester, it was immediately hydrolyzed in alcoholic potash under reflux for 3 hrs. After diluting with water, the non-acidic impurities were extracted as before. The aqueous layer is extracted with ether after acidifying with dilute HCl. After drying and removing solvent, C22 acid was isolated. Recrystallization from 80% EtOH/water three times gave pure trans acid.

mp 181-2° C

λ max 376 mμ ($e = 46{,}400$); νmax 1,680 cm^{-1} (carbonyl) 269 mμ ($e = 11{,}700$)

δ($CDCl_3$):

In the above synthesis, equal mole quantities of retinal, sodium hydride and triethylphosphonoacetate were employed although this exact ratio is not necessary.

C22 acid and its salts can be applied in the form of an ointment, as a solution in oil or as a powder. In each instance a concentration of about 1% has been found suitable although larger or smaller concentrations may be used. Below about ½%, the effectiveness falls off and increasing the concentration from 1 to 2% increases the effectiveness only slightly. Therefore a concentration of about 1% whether in an ointment, oil solution or powder is about optimum.

Suitable oil carriers include physiologically acceptable oils in which the acid is soluble such as isopropyl myristate, corn oil, cotton seed oil and the like. Powder can be prepared utilizing the C22 acid crystals by grinding the crystals with a suitable inert carrier such as talc. C22 acid or its salts can be combined with any of the usual ointment bases used in pharmacy. One suitable base is known as NIB (non-ionic base) developed by the University of California School of Pharmacy having the following approximate composition:

Cetyl alcohol	6%
Stearyl alcohol	6
White petrolatum	14
Liquid petrolatum	20
Methyl paraben	0.15
Propyl paraben	0.06
Polysorbate 80	1.5
Polyoxyl 40 stearate	5
Propylene glycol	2
Purified water	q.s. 100%

In testing the compound of the present invention and comparing it with its homologs, male rats weighing 230 to 240 grams were anesthetized with ethyl ether and the hair on the back was shaved off. An incision 6 cm in length was made through the skin and cutaneous

muscle at a distance of about 1.5 cm from the midline on each side. No ligatures were used and bleeding usually ceased after a few minutes. The incisions were closed with continuous through and through sutures with stitches 0.5 cm apart. The wounds were left undressed. NIB preparations were applied, with gentle rubbing, directly on the sutured wound right after wounding. The application was repeated, once a day, on the first and second days after wounding. For the control, only NIB was applied. On the seventh day after wounding, the tensile strength of the wound was measured with a tensiometer by cutting the sutures with a pair of scissors and measuring the tension necessary to pull the wound open. The tensile strength required for opening the wound was measured in grams. In this series of tests, one group of animals was treated with plain NIB base while other groups were treated with various acids in NIB base. The following results were obtained:

EFFECT OF TOPICAL APPLICATION OF RETINOIC ACID HOMOLOGS ON TENSILE STRENGTH OF HEALING WOUND

Group	No. of animals	Compound applied (acid)	Tensile strength
I	14	NIB	451 ± 9
II	6	1% C_{12}	470 ± 10
III	8	1% C_{15}	472 ± 7
IV	11	1% C_{17}	462 ± 6
V	12	1% C_{20}	537 ± 4
VI	6	½% C_{22}	521 ± 3
VII	8	½% C_{24}	481 ± 2

All of the acids had the formula 2,6,6,-trimethyl-1-X-cyclohex-1-ene, wherein X is a side chain of conjugated double bonds. The C20 acid is, of course, retinoic acid and C22 is the acid of the present invention.

In another series of tests, granulation was measured by the cotton pellet method.

This method involves subcutaneous implantation of cotton-pellets and measuring the size of the granuloma induced after a few days. Anti-inflammatory agents reduce the size or weight of granuloma as compared with that of the control. Those compound derivatives which promote healing increase the size or weight of the granuloma.

Growth of granulation tissue into cotton-pellets was induced by subcutaneous implantation at two symmetrical dorsolateral sites of Sprague-Dawley male rats weighing 120 ± 5 g under ether anesthesia.

The cotton-pellet implanted on the right side contains the compound under test and the cotton-pellet implanted on the left side serves as the control. The compound was introduced to the pellet as its ether solution. The ether was completely evaporated before implantation. On the seventh day after implantation the animals were killed with ether and the body weights were taken. The granulomas were carefully removed and weighed rapidly on a torsion balance. After drying in an oven at 65° C for 48 hours the dried slices were weighed again.

EFFECT OF RETINOIC ACID HOMOLOGS ON COTTON-PELLET INDUCED GRANULOMA

group	No. of animals	comp'd. applied	body wt. change ave. g.	WET expt.	WET control	granuloma wt. mg. expt. control
I	6	C_{12}	+48	213.8±7.5	215.3±5.1	1.0
II	6	C_{15}	+38	214.5±2.3	215.4±5.0	1.0
III	12	C_{17}	+50	220.5±8.2	218.9±6.0	1.0
IV	12	C_{20}	+50	332.2±4.9	218.7±2.5	1.5
V	12	C_{22}	+47	438.0±5.4	225.9±7.4	1.9
VI	10	C_{24}	+46	302.0±12.7	216.5±6.9	1.4
group				DRY		
I				28.3±3.1	26.8+2.9	1.0
II				27.6±1.0	27.8±0.8	1.0
III				30.2±2.7	29.9+3.0	1.0
IV				59.9±1.7	35.1±1.9	1.5
V				69.8±2.6	36.1+2.5	1.9
VI				47.4±2.1	31.3+2.2	1.5

In addition to the C22 acid itself the salts of the acid were found to be effective. The salts of any physiologically acceptable metal can be employed.

In the following example 2 mg of the sodium salt of the C22 acid was employed. The cotton pellet method described in detail above was used and the following results obtained:

Wet			Dry		
Exp.	Control	Ratio	Exp.	Control	Ratio
332.1	207.3	1.7	40.0	22.7	1.8

I claim:

1. A wound healing composition comprising an effective amount of a compound selected from 2,6,6,-trimethyl-1-(10'-carboxy-3', 7'-dimethyldeca-1', 3', 5',7', 9',-**pentaenyl)-cyclohex-**1-ene acid and its salts in a physiologically acceptable topical carrier selected from the group consisting of an oil, an ointment or a powder.

2. A method of healing a wound comprising applying to said wound site a composition of claim 1 containing about 1% of the acid or its salt.

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