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Chemoprophylactic agents in schistosomiasis:* eremanthine, costunolide, α-cyclocostunolide and bisabolol

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The wood oils of the trees *Eremanthus elaeagnus* Sch.-Bip., *Vanillosmopsis erythropappa* Sch.-Bip. and *Moquinea velutina* Bong. inhibit penetration of cercariae of the trematode *Schistosoma mansoni*. The effect is due to the presence of α,β-unsaturated sesquiterpene lactones of which three, eremanthine (I), costunolide (III) and α-cyclocostunolide (V), have been characterized. The sesquiterpene alcohol (−)-bisabolol (II) present in two of the oils is weakly active while dihydro-β-cyclocostunolide (VI) which lacks the unsaturated lactone function is inactive. I and V react with the sulphydryl group of cysteine, and their activity may be related to inhibition of sulphydryl groups in cercarial enzymes.

Animals may be protected against infection by cercariae of *Schistosoma mansoni* by application of certain essential oils to the skin (Pellegrino, 1967). Among active oils are those of a number of Coniferae, for example of the wood of *Juniperus virginiana* L. the Eastern red cedar (Campbell & Cuckler, 1961), and of Leguminosae, for example, the fruit oil of *Pterodon pubescens* Benth. (Mors, Fascio & others, 1967). Active compounds have been shown in many cases to be sesqui- and di-terpenes (Gilbert, de Souza & others, 1970a), and it thus seemed appropriate to examine oils of the family Compositae, known to be rich in sesquiterpenoid compounds. Three common Brazilian arboreal species were first studied, namely, *Eremanthus elaeagnus* Sch.-Bip., *Vanillosmopsis erythropappa* Sch.-Bip., and *Moquinea velutina* Bong. Hexane extracts of the heart- and sap-wood of *E. elaeagnus* and *M. velutina* were in fact found to afford complete protection against infection by *S. mansoni* (Gilbert, de Souza & others, 1970b). The hexane extract of *V. erythropappa* afforded partial protection only, but after distillation almost all cuts gave complete protection. These extracts were therefore subjected to chemical study, and the principal active components are now reported.

CHEMISTRY

The oil (192 g) obtained by percolation of the pulverized trunk wood of a mature tree *Eremanthus elaeagnus* (12 kg) with hexane (25 litre) at room temperature for 72 h

* Previous paper: Gilbert, de Souza & others (1970a).

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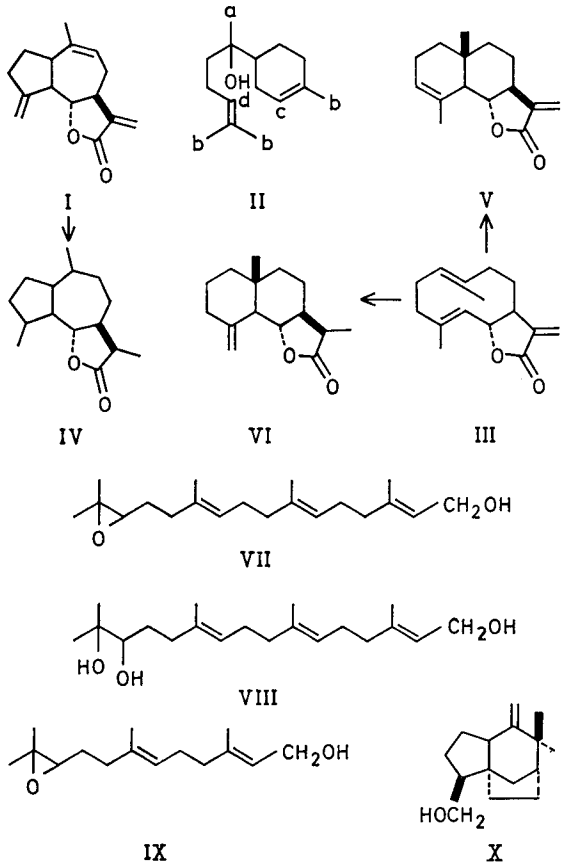

slowly deposited crystalline eremanthine (I, 25·7 g). After removal of the supernatant oil and recrystallization from hexane, eremanthine had m.p. 73–74°, $[\alpha]_D^{29}$ — 59° (c, 1% in chloroform).

Chromatography of the oily portion of the extract (25 g) over silica gel (350 g) in hexane and hexane–ethyl acetate 20:1, gave bisabolol (II, 11 g), identified with an authentic sample from *Vanillosmopsis erythropappa* by t.l.c., infrared and nmr spectroscopy.

Percolation of powdered trunk wood of *V. erythropappa* (20 kg) with hexane (30 litre) gave, after removal of the hexane under reduced pressure, a brown oil (400 g). Distillation of this oil (25 g) through a 120 cm approx. spinning band column at 139°/37 mm gave pure bisabolol (12 g, 48% yield, another 24% of crude bisabolol was also obtained), nmr (60 MHz, $CDCl_3$) 1·06 (3H, singlet, *a*), 1·63 (9H, singlet, *b*), 5·07 (1H, broad triplet, *c*), 5·30 (1H, broad singlet, *d*) δ; mass spectrum showed M^+ at 222.

The specimen was identical with authentic bisabolol by t.l.c., g.l.c., and infrared comparison (Gottlieb & Magalhães, 1958).

Late fractions were contaminated with a less volatile component, and the blue colour of the still residue suggested the presence of an azulene produced by thermal degradation of a guaianolide-type sesquiterpenoid. Two minor components were isolatable by silica-gel column chromatography of the original hexane extract (70 g).

Gradient elution with hexane, benzene and ethyl acetate gave successively bisabolol, friedelin, friedelanol (5 g), eremanthine (1·5 g) and a crystalline ester (1·0 g) m.p. 108°. Eremanthine, m.p. 73–74° was identical to that obtained from *E. elaeagnus* by t.l.c., nmr infrared and mixed m.p. comparison. The second ester, an α,β-unsaturated γ-lactone, was identified as costunolide (III) by comparison of infrared nmr and mass spectra (Rao, Kelkar & Bhattacharyya, 1960; Herout, Suchy & Sǒrm, 1961).

Eremanthine (I) was identified as an α,β-unsaturated γ-lactone by infrared and ultraviolet spectrometry. High resolution mass spectrometry showed the molecular formula to be $C_{15}H_{18}O_2$ (found: mol wt 230·13089; calc., 230·13067) and hydrogenation gave a hexahydroderivative identical to perhydro-dehydro-costuslactone (IV, Mathur, Hiremath & others, 1965) by infrared comparison (*see* Vichnewski & Gilbert, 1972).

The oil obtained by extraction of the trunk wood of *Moquinea velutina* yielded, by silica gel chromatography, two crystalline compounds. The less polar material (V), m.p. 80–82°, $[\alpha]_D^{24}$ + 116·5° ($CHCl_3$) shown to be identical with α-cyclocostunolide (Kulkarni, Kelkar & Bhattacharyya, 1964; Jain & McCloskey, 1969), while the second component, m.p. 135–137°, $[\alpha]_D^{25}$ + 165° ($CHCl_3$) was identical with dihydro-β-cyclocostunolide(VI) (Jain & McCloskey, 1969), both compounds previously derived from the naturally occurring costunolide (III). Identities of both compounds were established by ultraviolet, infrared, nmr and mass spectrometry and by direct chromatographic, spectral and mixed m.p. comparison with authentic specimens (Tomassini & Gilbert, 1972).

Using the method of Kupchan, Fessler & others (1970) it has been shown that α-cyclocostunolide and eremanthine, but not dihydro-β-cyclocostunolide, react with cysteine. The cysteine-α-cyclocostunolide addition product was shown to run as a separate spot, detected by ninhydrin, on a thin-layer of Sigmacell (acetone–water, 1:1).

PHARMACOLOGY AND DISCUSSION

The protection afforded by the oils and their components against infection by cercariae of *Schistosoma mansoni*, was assessed by application to the skin of mice. Eleven mice were used for each experiment, the substances being applied to the tails either pure or in hexane or acetone solution. After 24 h these mice were exposed for 45 min by the tail immersion method (Pellegrino & Katz, 1968), to 200 cercariae of *S. mansoni* (L.E. strain, Belo Horizonte) shed by laboratory reared and infected *Biomphalaria glabrata.* After seven weeks the mice were killed and the schistosomes present collected by perfusion (Pellegrino & Siqueira, 1956). Eleven control animals untreated or treated with pure solvent were exposed in the same way in each experiment. Protective activity is recorded in Table 1 as a comparison of the mean worm burden of treated mice with that of the controls.

From the results the protective action of *E. elaeagnus* and *V. erythropappa* oils may be largely ascribed to the α,β-unsaturated sesquiterpene lactone eremanthine (I) accompanied in the case of *V. erythropappa* by the related lactone, costunolide(III). Pure bisabolol however does afford a high degree of protection, though when diluted with inactive materials as in the crude oil of *V. erythropappa* this falls off sharply. With *M. velutina* the degree of protection against cercarial penetration is markedly lower with the pure component α-cyclocostunolide (V) than with the crude total hexane extract.

Table 1. *Worm burdens observed in mice treated with components of* Eremanthus Elaeagnus, Vanillosmopsis Erythropappa *and* Moquinea velutina *and and exposed to* Schistosoma Mansoni *cercariae.*

Material applied (extraction solvent)*	Concn % applied to skin (solvent)*	Mean as % of control	Worm burden Mean number of worms per animal (range) Treated	Control	Animals died Treated	Control
E. elaeagnus wood (H)	100	2·9	<1 (0–3)	15 (8–26)	2	2
E. elaeagnus wood (E)	100	74·2	14 (7–26)	19 (16–23)	2	6
V. erythropappa wood (H)	100	40·0	6 (0–17)	15 (9–26)	1	2
V. erythropappa wood (H) distilled†	100	0·0	0	25 (16–32)	4	3
M. velutina wood (H)	100	0·0	0	15 (8–26)	1	2
M. velutina wood (E)	100	0·6	<1 (0–1)	19 (8–31)	2	3
Bisabolol	100	3·1	<1 (0–2)	9 (2–18)	0	0
Eremanthine	50(A)	0·0	0	35 (10–66)	3	2
Eremanthine	10(H)	0·0	0	23 (15–28)	1	3
Eremanthine	1(H)	44·0	11 (1–17)	23 (15–28)	0	3
Eremanthine	0·1(H)	102·7	25 (20–38)	23 (15–28)	3	3
Costunolide§	10(B)	3·2	1 (0–6)	27 (5–60)	4	3
α-Cyclocostunolide	5(H)	12·8	3 (0–10)	26 (20–45)	1	1
Dihydro-β-cyclocostunolide	10(H)	83·9	36 (22–61)	43 (30–60)	0	2

* 100% means no solvent, H = hexane, A = acetone, B = benzene, E = ethanol. Controls were untreated, or treated with hexane or acetone respectively.

† Result reproducible with four major distillation fractions containing bisabolol and eremanthine (by t.l.c. and infrared spectrometry).

§ From *V. erythropappa.* Friedelin and friedelanol, also isolated, were inactive at 10% concentration.

Previously, protective activity of this type has been found in two diterpene alcohols —14,15-epoxygeranylgeraniol (VII) and 14,15-dihydro-14,15-dihydroxygeranylgeraniol (VIII) (Mors & others, 1967; Gilbert & others, 1970a)—and in a series of both sesqui- and di-terpene acids. Bisabolol (II) cannot be compared in activity to the alcohols VII and VIII which give total protection against infection when applied at 5–10% concentration. In activity it lies between the sesquiterpene alcohols, 10,11-epoxyfarnesol (IX, worm burden 26% of control at 10% concentration) and khusimol (X, worm burden 58% of control at 10% concentration). On the other hand, eremanthine (I) has activity comparable to the previously recorded diterpenes and is the first lactone to exhibit such activity.

Table 2. *Chymotrypsin-like activity of cercarial penetration enzyme in the presence and absence of eremanthine, at 37°.*

Substance	% Activity on ATEE[1] α-Chymotrypsin	Cercarial enzyme[2]
Control	100	100
Control + methanol (20 μl)	95	90–93
Eremanthine (100 μg) in methanol (20 μl)	55	56–60
Eremanthine (200 μg) in methanol (20 μl)	—	46

[1] The reaction mixture consisted of 0·5 ml of 0·005 *N*-acetyl tyrosine ethyl ester (ATEE) buffered at pH 7·9, 0·4 ml of 0·05 M phosphate buffer and 0·1 ml of either cercarial enzyme or chymotrypsin solutions. After 5 min incubation the reaction was stopped by addition of 50% trichloroacetic acid. A control was run in the presence of methanol the solvent for eremanthine.

[2] Purified by the method of Gazzinelli, Ramalho Pinto & Pellegrino (1966).

The difference in activity between the α,β-unsaturated lactone V and the saturated lactone VI leads us to believe that the unsaturated lactone function is responsible for the effect. The presence of the same function has been related (Kupchan & others, 1970; Hanson, Lardy & Kupchan, 1970) to the anti-tumour activity of a number of similar but more polar sesquiterpene lactones and is attributed to the ability of the α,β-unsaturated lactone to add the terminal -SH function in cysteine or other residues, with resulting inhibition of certain enzymes. The lactones I and V (but not VI) in fact react with cysteine in the same way. Penetration inhibition may thus be related to the inhibition either of the penetration enzymes, or of an enzyme within the cercaria or newly-formed schistosomulum. This aspect is presently under investigation, and preliminary results are reported in Table 2, where partial inhibition of the chymotrypsin-like activity of cercarial penetration enzyme by eremanthine is shown. Esterolytic activity of both enzymes was determined according to Hestrin (1949) using ATEE as substrate.

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