moval of the medial wall of the middle ear. The tegmentum vasculosum was separated from the other cochlear structures with fine needles under 60-fold magnification. The membranous structures of the guinea pig cochlea were lifted from the bony modiolus after careful removal of the bony capsule. After the membranous structure was stretched on a glass slide, the stria vascularis from the second turn was removed from the ligamentum spirale with a hair mounted in glass. After homogenization in twice-distilled water (0.05 to 0.2 mg/100 μl, dry weight), the tissues were assayed as described by Bonting (*9*).

The CMP, obtained after acoustic stimulation by pure tones (0.2 to 10.0 kc/sec, 80 db), was recorded from a nichrome wire electrode implanted in the scala vestibuli of the first turn of a guinea pig anesthetized with nembutal, and an indifferent electrode implanted in the neck muscles. After amplification, the electric signal was displayed on an oscilloscope. The perilymphatic space (scala vestibuli) of part of the first and second turns was perfused with 10 μl of modified Krebs-Ringer solution per minute (*10*) with or without ouabain (10^{-8} to $10^{-3}M$) or erythrophleine ($10^{-5}M$). The emerging perfusate was immediately removed by suction so that entry of drug into the blood circulation would be avoided. The amplitude of the CMP was measured on the oscilloscope screen before perfusion and at various intervals after the start of perfusion.

In agreement with Iinuma's findings (*11*) we found in total membranous structures of the cochlea considerable Na^+- and K^+-activated adenosine triphosphatase activity sensitive to ouabain (Table 1). Our separate assays of stria vascularis from guinea pig and chicken tegmentum vasculosum [comprising stria vascularis and Reissner's membrane and shown to have a secretory function (*12*)] showed a remarkably high activity of this enzyme. The time course of the inhibitory effect of ouabain on the CMP is presented in Fig. 1. The inhibition of the CMP was dependent on the concentration of ouabain; erythrophleine produced a slightly smaller effect (Fig. 2). The inhibition was reversible, since after the perfusion of ouabain was discontinued, the CMP amplitude gradually increased again.

The presence of a high activity of Na^+- and K^+-activated adenosine triphosphatase that is sensitive to ouabain, particularly in the tegmentum vasculosum and the stria vascularis, and the abolition of the CMP by ouabain and erythrophleine strongly suggest the operation of a Na^+- and K^+-activated adenosine triphosphatase cation pump system in the generation of the cation gradients required for cochlear function.

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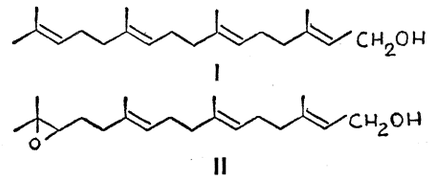
Chemoprophylactic Agent in Schistosomiasis: 14,15-Epoxygeranylgeraniol

Abstract. *The occurrence of all*-trans *(−)-14,15-epoxygeranyl-geraniol in* Pterodon pubescens *Benth. is established, and its prophylactic activity against infection by* Schitsosma mansoni *demonstrated. Two other diterpenes present in the oil are inactive.*

Certain essential oils exhibit prophylactic activity in schistosomiasis. Such a property has been attributed, for example, to North American cedarwood oil (*1*), which provides protection against the penetration of cercariae of *Schistosoma mansoni* in mice. These results stimulated the search for essential oils of Brazilian plants which might exhibit similar activity. As literature reports (*2*) indicated that it might resemble cedarwood oil in composition, attention was directed to the oil of the fruits of *Pterodon pubescens* Benth. (fam. Leguminosae Papilionoideae), commonly known as "sucupira branca." We have been unable to confirm the reported chemical similarity between the two oils, but have shown (*3*, *4*) that the *Pterodon* oil, pure or diluted, when applied topically to the tails of mice shows outstanding protective action against the penetration of cercariae of *S. mansoni* (Table 1).

Extraction of freshly collected fruits of *P. pubescens* (1 kg) with hexane gave, after evaporation of the solvent, a viscous, brown oil (100 g). Chromatography of the oil (100 g) on silica gel (2 kg), by elution with hexane and then hexane-acetone (20 : 1) gave successively geranylgeraniol (I), (−)-14,15-



epoxygeranylgeraniol (II), and a crystalline diterpene. Geranylgeraniol, purified by distillation and preparative thin-layer chromatography, was obtained as a colorless oil, b.p. 110°C at 0.01 mm; and identified by infrared (*5*) and thin-layer chromatographic comparison (*6*) with an authentic sample (*7*). The nuclear magnetic resonance (NMR) spectrum showed absorption at 1.60 [9 H (this notation indicates nine protons), singlet, three allylic methyl groups], 1.68 (6 H, singlet, two allylic methyl groups), 2.00 and 2.05 (sharp, allylic methylene), 4.15 [2 H doublet: *J* (spin-spin coupling constant) 7 hz; CH_2 OH] and 4.90 to 5.70 parts per million (ppm) (4 H, multiplet, vinyl protons), and was identical with that of geranylgeraniol from linseed oil (*5*) and from *Cedrella toona* Roxb. (*8*), as well as being compatible with an all-*trans* structure (*9*). The high-resolution mass spectrum (*10*) showed M+ (molecular ion) at 290.26094 (calculated for $C_{20}H_{34}O$, 290.26095), and M−H_2O at 272.25042 (calculated for $C_{20}H_{32}$, 272.25039). Principal fragmentation peaks were observed at *m/e* (ratio of mass to charge) 221 (M−69), 203 (M−69−18), 191 (M−69−30), 189, 161, 147 to 149, and 135 to 137. Geranylgeraniol proved inactive toward cercariae of *S. mansoni* (Table 2). (−)-14,15-Epoxygeranyleraniol (II), purified by distillation and preparative thin-layer chromatography, was obtained as a colorless oil

Table 1. Protective action of the crude oil of the fruits of *Pterodon pubescens* against infection by *Schistosoma mansoni*. The oil was applied in solution (solvents and concentrations indicated) to the tails of mice, and, after 24 hours, these were exposed to 100 cercariae (*16*). After 7 weeks, the schistosomes were collected by perfusion (*17*). DMSO, dimethyl sulfoxide.

Dilution (%)	Solvent	Animals (No.)	Schistosomes per animal (Av. No.)
25	Ether	6	0.0
5	Ether	8	.6
25	DMSO	11	.3
5	DMSO	11	1.4
(Untreated animals)		11	26.4

Table 2. Protective action of the crude oil of the fruits of *Pterodon pubescens*, and of its constituents, against infection by *Schistosoma mansoni*. Technique as in Table 1. The substances were applied as 20 percent solutions in hexane to the tails of mice, and, after 24 hours, these were exposed to 200 cercariae. After 7 weeks, the schistosomes were collected by perfusion.

Substance	Animals (No.)	Schistosomes per animal (Av. No.)
Crude oil of *P. pubescens*	9	0.5
Geranylgeraniol	8	45.0
14,15-Epoxygeranylgeraniol	8	0.0
Diterpene $C_{24}H_{32}O_6$	7	64.7
None*	8	64.7

* Untreated animals were controls.

Table 3. Protective action of 14,15-epoxygeranylgeraniol, in various dilutions, against infection by *S. mansoni*. Technique as in Table 1, except that the animals were exposed to 150 cercariae and killed after 35 days.

Concentration	Animals (No.)	Schistosomes per animals (Av. No.)
1 percent in ether	10	6.5
5 percent in ether	10	0.0
10 percent in ether	10	.0
Not diluted	10	.0
Control*	10	28.2

* Untreated animals were used as controls.

with $[\alpha]_D^{26}$ equal to $-1.92°$; n_D^{21} equal to 1.4955; and the boiling point being 120°C at 0.01 mm. The infrared spectrum, similar to that of compound I, is distinguished by absorption at 873 cm^{-1} (broad, medium epoxide) and by weaker absorption in the 830-cm^{-1} region than that of compound I (*5*). The NMR spectrum showed absorption at 1.24 and 1.28 [two 3 H singlets, $(CH_3)_2$ C(OR)-C], 1.59 (6 H, singlet, allylic CH_3), 1.66 (3 H, singlet, allylic CH_3), 1.98 (1 H, broad singlet eliminated by D_2O, O*H*), 2.03 (12 H, multiplet, allylic CH_2), 2.67 (1 H, triplet; *J*, 6.5 hz; epoxide *H*), 4.09 (2 H, doublet; *J*, 6.5 hz; $=CH{\cdot}CH_2OH$), 5.10 (2 H, broad, vinyl *H*), and 5.36 ppm (1 H, triplet; *J*, 7 hz; vinyl *H*). The upfield shift, relative to compound I, of two methyl absorptions, which are no longer allylic but whose relatively low-field position indicates attachment to an oxygen-bearing carbon atom when it is considered in conjunction with the appearance of the one-proton triplet at 2.67 ppm, and the disappearance of one vinyl proton absorption is entirely in accord with the terminal-epoxide structure. Apart from these differences, the NMR spectrum is closely similar to that of compound I, and, with the absence of ultraviolet absorption, leads to structure II (*11*), in which the double bonds have the all-*trans* configuration. Confirmation was obtained by high resolution mass spectrometry (*10*), which showed M^+ at 306.25487 (calculated for $C_{20}H_{34}O_2$, 306.25587) and $M-H_2O$ at 288.24461 (calculated for $C_{20}H_{32}O$, 288.24530). Fragmentation peaks occurred at *m/e* 274 to 276, 189, 175, 161, 153, 135, 121, and 107. 14,15-Epoxygeranylgeraniol reproduces the biological activity of the crude oil (Tables 2 and 3). The crystalline diterpene, $C_{24}H_{32}O_6$ (found: C, 69.31 percent; H, 7.56 percent; molecular weight by mass spectrometry, 416; calculated for $C_{24}H_{32}O_6$: C, 69.21 percent; H, 7.74 percent; molecular weight, 416), m.p. 220 to 221°C; $[\alpha]_D^{26}$ $-62°$ (*c*, 1.0, in chloroform), characterized as a tetracyclic aldehyde containing two acetoxyl groups and an α, β-disubstituted furan ring, is the object of present studies. It is inactive towards cercariae of *S. mansoni*.

The chemical similarity of epoxygeranylgeraniol (II) to substances recently found to have juvenile-hormone activity in insects, such as methyl-10,11-epoxyfarnesoate (*12*), raises the question as to whether a similar mechanism is not involved in its action. In the case of protection with the crude oil of *Pterodon pubescens*, it has been established that about 40 percent of the cercariae do penetrate the skin of the host animal, but do not develop into adult worms (*3*, and subsequent unpublished work). Farnesol, which was included in the tests because of its chemical similarity and known (albeit small) juvenile hormone activity, was inactive. On the other hand, farnesoic acid showed pronounced activity (*13*). It has been suggested (*14*) that the true juvenile hormones may be monocyclic, not open-chain, terpenes. The difference in activity between the readily cyclized, terminal epoxide II (*15*) and geranylgeraniol (I) points to a similar possibility for the two constituents of *Pterodon* oil.

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