Our results on protein yield from (1) was in agreement with those reported in the literature (6 mg per eye in the present study compared to 8 mg per eye in Berman[6]). Moreover, Lowry protein determination showed that (2) yielded more than eight times more total protein (mg protein/RPE) than (1). This suggests that (2) has dislodged more RPE cells into the buffer than (1) therefore resulting in a higher protein yield. This appears contrary to our histological examinations that (1) yields more 'intact' RPE cells. One possible explanation is that a significant number of RPE cells collected from (2) were disrupted (i.e. not 'intact' cells) as a result of vortexing thus lowering the number of intact RPE cells from (2). The protein yield, nevertheless, suggests that (2) collected more proteins from the bovine RPE than (1).

The retinyl ester hydrolase activity has been reported in the retinal pigment epithelium of human cells[7]. In addition, the enzymatic activity was shown to be stereospecific, i.e. more active towards hydrolysis of 11-*cis* than all-*trans* substrate. In this study, our results agree with the literature that RPE protein contains active enzymes to hydrolyze both 11-*cis* and all-*trans* retinyl palmitate (table 2). Moreover, these enzymes were found to favor hydrolysis of 11-*cis* over the all-*trans* substrate. It is important to note that REH activity was found in RPE cells collected by both methods employed in this study and that RPE cells collected by (1) possessed higher specific activity for both 11-*cis* and all-*trans* retinyl palmitate yet (2) yielded more total enzyme activity.

In conclusion, both methods employed in the present study collected RPE cells of good quality. Although (1) resulted in relatively more pure RPE cell preparation, (2) yielded more RPE proteins and higher overall enzyme activity. Consequently, we suggest that the method tested in the present study (2), offers a new and convenient approach to collect more RPE protein appropriate for certain investigations such as enzyme purifications.

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Urushiol components as mediators in DNA strand scission

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Summary. Poison oak urushiol, a mixture of 3-alk(en)ylcatechol derivatives was found to mediate DNA strand scission in the presence of oxygen and with copper(II) chloride as a catalyst. The reaction is believed to occur via activated reduced oxygen produced during oxidation of the catechol into its *o*-quinone derivative.
Key words. DNA strand scission; urushiol; poison oak; 3-alk(en)ylcatechol.

The ability of plant secondary metabolites to mediate DNA strand scission has been recognized recently[1]. DNA cleaving compounds are of special interest for their possible applications in molecular biology, and in the development of novel chemotherapeutic agents[2]. Plant natural products shown to cleave DNA include quinones like daunomycin[3], and polyphenolic compounds like 5-alkylresorcinols[4]. Daunomycin cleaves DNA in the presence of a reducing agent and iron. Resorcinol derivatives require copper salts and oxidation by molecular oxygen for activity. A mechanism involving a catechol copper complex as the active species has been proposed. These results suggested that urushiol resins from poison oak (*Toxicodendron diversilobum* (L.) Kuntze) or poison ivy (*T. radicans* (T. and G.) Greene) are likely to cleave DNA as well. Urushiols are the principal cause of contact dermatitis in North America[5] and have been studied extensively for sensitizing and irritating properties, but not for antineoplastic activity. Therefore, we set out to determine if these natural products can mediate strand scission of Simian virus 40 DNA.

Materials and methods

Extraction and separation. Poison oak stems were collected in March 1989 at the UCI Wildlife Conservation Area, Irvine, California, and extracted immediately after collection, as described by Dupuis[6]. Stems (2 kg) were extracted with ethanol (12 l) in a Waring blender at room temperature. Evaporation of the solvent afforded 75 g of crude extract.

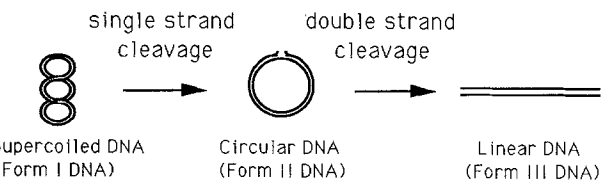
The crude extract of poison oak was separated on column chromatography twice over silica gel eluted with petrol and petrol-EtOAc (95:5), then over cross-linked PVP eluted with EtOAc. Fractions were analyzed by silica gel TLC developed in petrol-EtOAc (8:2) and visualized by spraying with 5% $FeCl_3$ in EtOH and with 5% vanillin-5% H_2SO_2 in EtOH. A purified urushiol extract was identified by the IR, UV and NMR spectra[6].

Components of the urushiol extract, heptadecylcatechol and unsaturated analogs, were separated as described elsewhere[7] with C18 reverse phase HPLC (Adsorbosphere C18, 10 µm, 250 mm × 10 mm ID) and MeOH-water (92:8) mixture at 7.5 ml/min with UV detection at 254 nm. Urushiol components (fig. 1) collected were characterized as heptadecylcatechol (**1**), heptadecenylcatechol (**2**), heptadecadienylcatechol (**3**) and heptadecatrienylcatechol (**4**) by comparison of NMR, UV and MS spectra and high performance TLC RF (Whatmann MKC18 reversed phase TLC) with the reported spectra and R_f values (the urushiol from poison oak (*T. diversilobum*) has been well studied and contains a mixture of four heptadecylcatechols as opposed to other *Toxidendron* species which produce the pentadecyl derivatives)[7].

DNA strand scission. Urushiol-mediated DNA strand scission was conducted similarly to that previously described by Barr et al.[8]. To 0.8 µmoles (resp. 0.4 µmoles) of each urushiol component in 50 µl of water/dimethoxyethane (8:2) was added 50 µl of 0.1 N NaOH. After 2 min at room temperature the solutions were treated with 50 µl of 30 µM (resp. 15 µM) aqueous $CuCl_2$ and aerated for 50 min with a stream of air. The solutions were finally neutralized with 50 µl of 0.1 N HCl. A 15-µl sample of each of the neutralized solutions was added to 200 ng of SV40 DNA in 25 µl of 50 mM sodium cacodylate, pH. 7.4. The DNA reaction mixtures were maintained at room temperature for 30 min, and then quenched by addition of 10 µl of 5 mM EDTA, in H_2O-glycerol (6:4) with 0.3% bromophenol blue and 0.4% SDS. The reaction mixtures were then analyzed by horizontal electrophoresis on a 1.2% agarose gel containing 1 µg/ml of ethidium bromide in 40 mM Tris buffer, pH 7.8, containing 5 mM NaOAc and 1 mM EDTA at 65 volts for 3 h.

Results and discussion

The property of urushiol and its congeners to mediate DNA strand scission was characterized by their ability to convert supercoiled ccDNA (form I DNA) to nicked circular DNA (form II DNA), and double stranded linear DNA (form III DNA) (scheme 1). Oxygen-treated urushiol components without the presence of copper ion seem to slightly degrade DNA. With copper present during the activation step, a marked, concentration-dependent, cleavage of DNA is seen. The solutions containing 0.06 µmole of urushiol component (diluted from 0.8 µmole) gave total cleavage of form I into form II,



Scheme 1. Cleavage of supercoiled DNA.

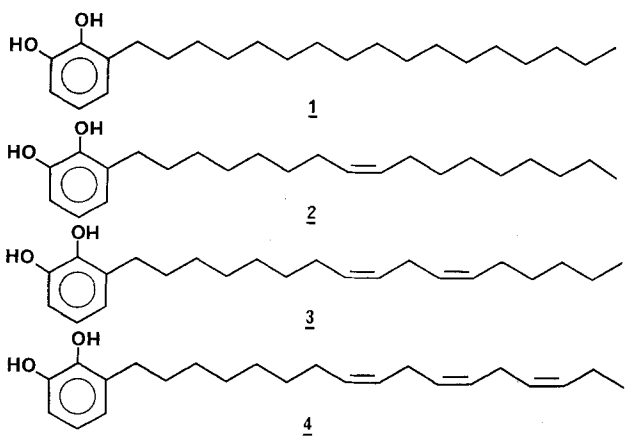


Figure 1. Structure of poison oak urushiol components.
1 3-n-Heptadecylcatechol
2 3-n-Heptadecenylcatechol
3 3-n-Heptadecadienylcatechol
4 3-n-Heptadecatrienylcatechol

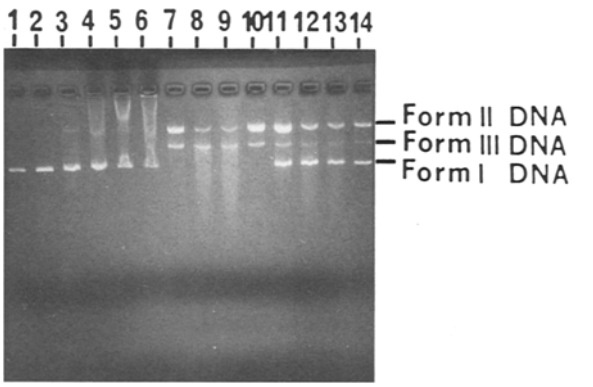
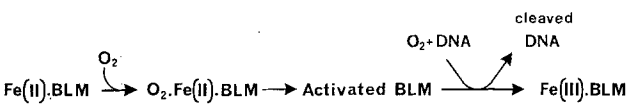


Figure 2. Strand scission of SV40 DNA.
1 untreated DNA; 2 DNA treated with $CuCl_2$ only; 3–6 DNA treated with 0.09 µmoles of oxygenated urushiol components without copper catalysis; 7–10 DNA treated with 0.06 µmoles of oxygenated urushiol components catalyzed with 0.012 µmole of copper chloride; 11–14 DNA treated with 0.03 µmoles of oxygenated urushiol components catalyzed with 0.06 µmole of copper chloride.



Scheme 2. Mechanism of DNA-cleaving activation of iron-bleomycin (Fe.BLM)[8].

indicating single strand scission, and form III DNA indicating double strand scission. The solutions containing 0.03 µmole of urushiol component (diluted from 0.4 µmole) gave partial cleavage of form I DNA (fig. 2). No differences of activity were seen between the different urushiol components with bleomycin (BLM), activated by various metals, as a mediator[1]. Recent studies suggest that bleomycin and its analogues act by site-selective binding to DNA and oxygen mediated scission of the strands. The mechanism of cleavage is believed to involve activated oxygen, produced as a consequence of oxidation BLM-chelated Fe(II) to Fe(III)[9]. In the case of urushiol, copper catalyzed oxidation of the catechol moiety can lead to the production of activated oxygen which, as described for BLM-Fe, is able to induce DNA breakage (scheme 2). Preliminary experiments show that similar DNA strand scissions can be caused by geranyl- and geranylgeranyl hydroquinone, both dermatotoxic compounds occurring in trichomes of species of *Phacelia* (Hydrophyllacea)[10].

Due to the strong skin irritation and allergic contact dermatitis, elicited by urushiol in vivo, it is somewhat difficult to investigate the actual occurrence of DNA cleavage by urushiol. However, these results suggest that it would be interesting for molecular biologists to design and synthesize a molecule structurally related to urushiol containing a DNA sequence specific recognition pattern[10] linked to a catechol unit through variable length hydrocarbon chain.

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Effects of isoxazolyl-naphthoquinoneimines on growth and oxygen radical production in *Trypanosoma cruzi* and *Crithidia fasciculata*[1]

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Summary. Several 4-(aminomethylisoxazolyl)-1,2-naphthoquinones inhibited growth and DNA synthesis in *Trypanosoma cruzi* and stimulated O_2 uptake and O_2^- generation by the parasite epimastigotes and their mitochondrial and microsomal membranes; these results support the idea that oxygen radicals play a role in quinone toxicity. Maximal effects on respiration and O_2^- generation were observed with antimycin-inhibited cells. Similar results as well as stimulation of H_2O_2 production were obtained with *Crithidia fasciculata* despite the presence of catalase in this organism.
Key words. Trypanosoma cruzi; *Crithidia fasciculata*; isoxazolyl-naphthoquinoneimines; growth inhibition; DNA synthesis; O_2^- production; H_2O_2 production.

Despite its epidemiological importance, the chemotherapy of Chagas' disease is still an unsolved problem[2]. Many drugs have been assayed for their action on *Trypanosoma cruzi*, the agent of Chagas' disease, but screenings did not include quinoneimines[3,4]. These compounds have similar chemical properties to quinones, including the ability to undergo one electron reduction to give a semiquinone-type free radical and two electron reduction to an aminophenol[5]. Fernandez et al.[6] synthesized a series of isoxazolyl-naphthoquinoneimines (fig. 1), on the assumption that the biological activity of the substituted isoxazol would enhance that of the naphthoquinone group. Therefore, it seemed of interest to establish whether the new compounds affect *T. cruzi* in