The techniques for the thalline color test and crystal tests as outlined by HALE[11] were used. The lichen substances were extracted from thallus tips with warm acetone. 2 or 3 extractions of each shock group and the control were prepared for each reagent used in the microcrystal tests. All of the tests for each culture group were done in time sequence. The slides were observed immediately after the reaction and at periodical intervals up to 6 months time. The reactions were immediate and stable, and no new substances formed during this period.

Results and discussion. Thalli of the shocked lichens appeared tan to khaki in color compared to the white color of the control lichens. The noted color change was observed several days after shock. No other morphological changes were observed in the shocked plants.

The thalline tests were performed on specimens consisting of half a lichen thallus and were observed under low magnifications. The results of the thallus tests agree with those listed by THOMSON[8] for *C. impexa*. There were no changes in the color tests after shock.

The results of the microcrystal tests were compared with pictures and descriptions of crystalline acids as given by ASAHINA[12], EVANS[13], HALE[11,14] and THOMSON[8]. 3 reagents, namely, glycerin-acetic acid, 1:1 (GE), glycerin-95% alcohol-water, 1:1:1 (GAW), and glycerin-alcohol-aniline 2:2:1 (GAAn) gave positive crystal tests for all groups. No crystals were observed with glycerin-alcohol-*o*-toluidine, 2:2:1 (GA*o*-T), or glycerin-alcohol-quinoline, 2:2:1 (GAQ). The results of crystal tests are indicated in the Figure. Row 1 (A, E, I) represents crystals occurring in the control lichens, row 2 (B, F, J) 2 weeks post shock, and rows 3 and 4 (C, D, G, H, K) 5 weeks post shock. All those in column 1 were treated identically with GE reagent, whereas those in column 2 were treated with GAW and column 3 with GAAn.

ASAHINA's microcrystal tests[12] are useful in determining which substances occur in the thallus. An attempt was made to standardize this technique in the present study by holding the slides one inch above the flame and to remove them as soon as a bubble formed. The slides were air cooled.

The predominant acids in the control were usnic and atranorine. It is possible that the concentration of these acids was depressed after shock, thereby permitting the detection of other substances. Perhaps in the present study, various reactions, such as oxidations, recombinations and other reactions[15] were initiated by shock. However, such substances which were observed after shock treatment were not observed in the controls and are not typically found in *C. impexa* or presumably *C. pacifica*. *C. pacifica* must contain the same substance as *C. impexa* since apparently there is no contradictory information on this lichen. Therefore, the modification in the crystals are attributed to shock treatment[16].

Résumé. Une augmentation rapide de la pression produisant comme une rafale d'air dans un tube de choc fit apparaître des substances additionelles et des modifications de couleur dans les talles du lichen *Cladonia pacifica*.

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Anthraquinone in Plant Surface Waxes

The most abundant constituents of the surface waxes of plants are aliphatic compounds, for example hydrocarbons, esters, aldehydes, ketones, acids and alcohols[1]. Although terpenoid and steroidal compounds[2] are present in some waxes, few waxes are known to contain aromatic structures. We wish to report the occurrence of anthraquinone in a cuticular wax [*Lolium perenne* L. (perennial rye grass)].

Lolium perenne L. was grown in seed boxes under varying conditions of light and temperature, harvested and extracted with chloroform to remove the surface wax (0.1–0.2% by weight of the freshly harvested grass). Separation of the surface wax by thin layer chromatography (TLC) on silica gel with a solvent of 5% diethyl ether: light petroleum (40–60 °C) revealed a small spot on visualization with dichlorofluorescein, of Rf 0.16. This fraction was isolated by preparative TLC, and GLC analysis gave a component of retention Index (I) = 2150 on 5% Apiezon L at 235°; this component had I = 2430 on 3% OV-17 at 235°. GLC analyses at higher temperatures showed components of greater retention indices present in this fraction, and these are still under investigation.

GC/MS determination of the peak with retention Index = 2150 on Apiezon L on an AEI/MS 902 gave a parent ion at 208 *m/e* and fragments at 180, 152 and 76 *m/e*, which suggested anthraquinone. A sample of authentic anthraquinone was found to have similar chromatographic characteristics to those quoted above, and the UV-spectrum (λ_{max} 252.5 nm, 264 nm, 273 nm, 324 nm) of the compound from *Lolium perenne* L. was identical to that of anthraquinone. The extinction coefficients showed that anthraquinone accounts for 0.2% of the total wax.

In addition, the IR-spectrum of the total fraction, run as a potassium bromide disc, showed absorption at

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1678 cm^{-1} (C=O); 1589 and 1580 cm^{-1} (C=C aromatic); 1330 cm^{-1}, 1170 cm^{-1}, 810 cm^{-1} and 695 cm^{-1} identical to that of anthraquinone with additional bands at 720 cm^{-1} and 730 cm^{-1} due to $-(CH_2)_4-$ and a broad absorption at 3450 cm^{-1} (OH), indicating the presence of other aliphatic components.

To ensure that the anthraquinone was not a contaminant, all solvents used were carefully purified and all apparatus and filter papers were washed with purified solvent before use. The possibility that the anthraquinone was being leached from the inner tissues by the chloroform extraction was tested by dipping the grass leaves for ten seconds whilst ensuring that the cut ends did not come into contact with the solvent. This is the accepted procedure for removal of surface waxes[3] and the anthraquinone was found in this extract.

Anthraquinone has been isolated from the heartwood *Quebrachia lorentzii*[4] but its presence in a cuticular leaf wax has not been reported previously. Our present finding and the occurrence of aromatic hydrocarbons[5] in banana leaf suggest that aromatic compounds may play an important role in the leaf metabolism. It has been widely accepted that cuticular waxes prevent UV-radiation damage within the leaf and these aromatic substances may be the UV shielding components. An alternative role for anthraquinone relates to the protection of the leaf from insect attack. 5-Hydroxy 1,4 naphthaquinone from the tree *Carya ovata* deters the beetle *Scolytus multistriatus* from feeding[6] and NORRIS[7] has suggested that compounds with the necessary redox potential could enter the exposed chemoreceptors of the beetle and block its oxidative metabolic mechanisms. It may be that anthraquinone can also act as an insect repellent. We have failed to find anthraquinone in a number of dicotyledons and intend to compare their insect deterrent capabilities with that of *Lolium perenne* L.

Résumé. L'anthraquinone a été extrait pour la première fois de la cire cuticulaire d'une plante, le *Lolium perenne* L. Son identité a été confirmée par la spectrométrie de masse, la chromatographie en phase gaseuse et sur lames minces, la spectrométrie infrarouge et ultraviolette et sa fonction dans la cire est discutée.

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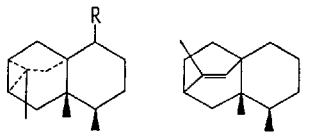
A Novel Tetracyclic Sesquiterpene from the Oil of Orejuela of *Cymbopetalum penduliforum* (Dunal)

'Orejuelas', the dried petals of *Cymbopetalum penduliforum* (Dunal) Baill. (Amonaceae), are well known in many parts of Central America and are used in Guatemala for flavoring pinol and other beverages[1]. Our interest in sesquiterpenes led us to examine the fragrant constituents of the petals. A literature search revealed the only species in this family that had been studied was *Anoma senegalensis*[2].

From a petroleum ether extract of the petals we have isolated[3] a tetracyclic sesquiterpene hydrocarbon **1** consisting of about 60% of the steam volatile portion of the extract. Elemental analysis indicated an empirical formula of $C_{15}H_{24}$[4]. High resolution mass spectral data confirmed the formula. An intense molecular ion $[M]^+$ 204 and a base peak at $[M\text{-}15]^+$ 189 were observed[5] suggestive of a polycyclic sesquiterpene structure[6]. The NMR-spectrum[7] showed absorptions at 0.8 (s, 3H), 1.15 (s, 3H), 0.7 (d, 3H), and 0.45 ppm (m, 1H) among others. No vinyl protons were detected. From these data we concluded that **1** contained at least 2 tertiary methyl groups, 1 secondary methyl group and 1 cyclopropyl proton. The IR-spectrum[8] showed absorptions characteristic of a saturated hydrocarbon. No UV-absorption above 220 nm was observed. Chemical evidence toward unraveling the structure of **1** supported spectral data. The compound was resistant to catalytic hydrogenation, could not be oxidized by perbenzoic acid and showed a faint yellow color with tetranitromethane[9]. The existence of a carbon-carbon double bond was ruled out by the spectral and chemical evidence. Thus **1** contained 4 rings of which at least one must be cyclopropyl.

When **1** was refluxed with cupric acetate in glacial acetic acid isomerization occurred to produce compound **2**. Preparative gas liquid chromatography (GLC) was used to purify the material. Mass spectral analysis indicated



1:R=H_2
3:R=O

2

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