

ISOLATION OF A CHLORINATED PIGMENT FROM GREEN SOIL*

By J. H. A. BUTLER†, D. T. DOWNING,‡ and R. J. SWABY†

Irregular olive-green patches have been found in lateritic and podzolic soils in certain areas of South Australia, Kangaroo Island, and Tasmania, apparently associated with decomposed roots of *Eucalyptus obliqua* L'Hérit. Extraction and purification procedures produced a crystalline red pigment and a wax. On the basis of elemental analysis, infrared, ultraviolet, nuclear magnetic resonance, and mass spectra the pigment is considered to be a hexachloro-polynuclear quinone, $C_{20}H_4O_5Cl_6$, of the dihydroxyperylenequinone or dihydroxydinaphthylquinone type. Compounds of these basic structures which have been isolated have usually been the products of fungi growing on the decomposing wood or roots of trees.^{1,2,3,4,5} In the present case attempts to isolate a pigment-producing organism were unsuccessful.

The wax associated with the pigment consisted mainly of a complex mixture of hydrocarbons resembling petroleum paraffins, together with esters of normal acids and alcohols from C_{12} to C_{30} .

Experimental

(a) *Extraction and Separation of Pigment and Wax*.—The soils were extracted continuously with cold or warm light petroleum (b.p. 60–80°) containing 2% acetic acid until no further colour was removed. In a typical experiment the extract from 2 kg soil was concentrated to 1700 ml, then extracted successively with several 500 ml portions of water, 2% $NaHCO_3$ in 30% ethanol, and boric acid–sodium borate buffer (pH 9) in 50% ethanol. The emerald-green buffer extracts were quickly separated from the light petroleum layer, extracted twice with fresh petrol, then acidified with dilute H_2SO_4 and the deep blue colloidal dispersion of pigment extracted with petrol (3 × 500 ml) to give a dark red solution.

The petrol solution from which the pigment had been extracted with buffer was evaporated to give 2.04 g neutral wax. The $NaHCO_3$ extract was acidified and extracted with petrol to give 0.12 g free acids.

(b) *Purification and Examination of the Pigment*.—The light petroleum solution of recovered pigment was subjected to countercurrent distribution between equal volumes of petrol and 96% aqueous methanol in eight separatory funnels. Complete double withdrawal gave 16 fractions of which fractions 4–8 (upper phase) yielded crystalline material on evaporation at 40°. The crystals were washed with light petroleum and recrystallized from benzene. A total of 7 kg soil gave 24 mg purplish black crystals with a brilliant bronze metallic lustre which were sparingly soluble in tetrachloroethane, chloroform, and benzene, less soluble in ether and petrol, and insoluble in ethanol, methanol, and water. The compound dissolved in concentrated sulphuric acid forming an intense emerald green solution, and was unstable in even weakly alkaline solutions. It decomposed without melting above 300°. Found: C, 47.3; H, 1.2; Cl, 37.9%; O, 14.3;

* Manuscript received March 13, 1964.

† Soil Microbiology Section, Division of Soils, CSIRO, Adelaide.

‡ Division of Organic Chemistry, CSIRO Chemical Research Laboratories, Melbourne.

¹ Calderbank, A., Johnson, A. W., and Todd, A. R., *J. Chem. Soc.* 1954, 1285.

² Anderson, J. M., and Murray, J., *Chem. & Ind.* 1956, 376.

³ Allport, D. C., and Bu'Lock, J. D., *J. Chem. Soc.* 1958, 4090.

⁴ Blackburn, G. M., Neilson, A. H., and Todd, A. R., *Proc. Chem. Soc.*, 1962, 327.

⁵ Batterham, T. J., and Weiss, U., *Proc. Chem. Soc.* 1963, 89.

active hydrogen, 0.27%; methoxyl, nil. λ_{max} . (CHCl_3 , dioxan) 225, 400, 550 $\text{m}\mu$, $\log \epsilon$ 5.73, 4.04, 4.40. After shaking with platinum and hydrogen the spectrum had λ_{max} . 230, 273, and 283 $\text{m}\mu$.

The infrared spectrum (tetrachloroethane, 0.2 mm cell) had strong absorption bands at 1620, 1425, 1370, 1140, 1090, and 955 cm^{-1} , and very weak absorption at 2900–2800, 1650, and 1530 cm^{-1} . The peak at 1620 cm^{-1} is attributable to an intramolecularly hydrogen-bonded, extended quinone system. The absence of hydroxyl absorption is to be expected in this type of system.⁶ A solution of the compound reduced with hydrogen on palladium showed peaks at 3390, 2900, and 1582 cm^{-1} and on reoxidation (H_2O_2) at 2900, 2840, and 1720 cm^{-1} .

The mass spectrum obtained by a special technique of heating the compound directly in the ion source, because of its extreme involatility, contained a group of peaks consistent with the isotope pattern of a molecule containing six chlorine atoms and a weight of 534 for the molecule containing only ^{35}Cl , so that the empirical formula is $\text{C}_{20}\text{H}_4\text{Cl}_6\text{O}_5$. Of equal magnitude was a group

TABLE 1
DIFFRACTION PATTERN OF POWDERED PIGMENT

dA	I/I ₁	dA	I/I ₁	dA	I/I ₁
13.9	20	4.623	20	3.225	80
12.9	20	4.446	10	3.202	40
11.2	60	4.314	80	3.156	60
9.67	60	4.202	40	2.982	70
8.65	20	4.121	10	2.933	30
6.857	20	3.878	20	2.873	60
6.595	40	3.725	60	2.742	60
6.215	40	3.658	40	2.578	20
5.937	50	3.540	100	2.532	20
5.317	10	3.485	100	2.473	30
5.053	40	3.366	50		
4.737	10	3.285	20		

of peaks having a Cl_4 isotope pattern with the ^{35}Cl -based peak at m/e 464, indicating that the parent ion readily lost two atoms of chlorine, although there was no peak corresponding to the loss of one chlorine atom. This was supported by the presence of a metastable peak which indicated that the chlorine elimination occurred by a concerted ionic mechanism. There was otherwise very little evidence of cracking, with the exception of a group of small peaks with a Cl_6 pattern having the ^{35}Cl -based peak at m/e 239, which suggests a doubly charged ion derived from the parent by loss of C_2O_2 . There was no peak at $(\text{M}-\text{CO})^+$. Two groups of small peaks near m/e 502 and 432 appeared to be due to an impurity with the formula $\text{C}_{26}\text{H}_7\text{O}_5\text{Cl}_5$ which also loses two chlorine atoms. Introduction of D_2O into the mass spectrometer increased the principal peaks by 2 mass units, indicating the presence of two exchangeable protons in the molecule.

The pigment was not sufficiently soluble in any solvent to obtain a satisfactory n.m.r. spectrum. When the spectrum was measured in tetrachloroethane solution at 60 Mc/s with tetramethylsilane as internal standard the only detectable absorption between –500 and 2000 c/s was at 444 c/s, but the solvent obscured the region from 350 to 375 c/s.

An X-ray diffraction pattern of the powdered pigment, using a 19-cm camera with $\text{Co } K_\alpha$ radiation gave the data set out in Table 1.

(c) *Examination of the Wax*.—The neutral wax (1.94 g) was saponified with 0.5N ethanolic KOH and separated into 1.24 g unsaponifiable material and 0.64 g acids.

⁶ Flett, M. St.C., *J. Chem. Soc.* 1948, 1441.

The unsaponifiable material was chromatographed on 50 g Florisil containing 10% water to give 0.73 g hydrocarbons eluted with light petroleum and 0.21 g alcohols eluted with 5% ether in light petroleum. The alcohols (200 mg) were separated into 160 mg of adducted and 40 mg of non-adducted material by treatment of a solution in light petroleum with urea moistened with methanol. The adducted alcohols were completely converted into acids by oxidation in acetone with $\text{CrO}_3/\text{H}_2\text{SO}_4$. The acids produced were converted into methyl esters by refluxing with methanol containing H_2SO_4 .

The acids obtained on saponification of the neutral wax were similarly converted into methyl esters, 600 mg of which were chromatographed on Florisil (10% water) from which 1% ether in light petroleum eluted 295 mg of non-polar esters. Increasing proportions of ether in petrol, pure ether, and ether-methanol mixtures produced no further discrete fractions in the resinous material gradually eluted.

Gas chromatography of the methyl esters of the acids on SE30 silicone gum at 280° showed a range of acids from C_{12} to C_{30} , with the even numbers predominating. The principal components were the C_{22} (13%), C_{24} (22%), and C_{26} (21%) acids. Similar results were obtained for the methyl esters of the acids derived from the long-chain alcohols.

The hydrocarbons were chromatographed at 260° and produced a "hump" between the retention times determined for C_{14} and C_{26} hydrocarbons, with superimposed peaks which appeared to belong to a homologous series in which compounds with odd and even numbers of carbon atoms were present in approximately equal amounts between C_{17} and C_{24} .

The authors are indebted to Dr. J. S. Shannon, Division of Coal Research, CSIRO, for determination of the mass spectra on an Atlas-Werke CH4 instrument, and to J. G. Pickering, Division of Soils, CSIRO, for X-ray diffraction data.