that part of the chloroform extract that it had taken up during the boiling was pipetted off. This distilled water extract was used for measuring the zone of inhibition of growth of *C. immitis* and for toxicity studies in mice.

One-tenth of a milliliter of this aqueous extract was placed on each of six testing disks and when dried these were placed in the center of 1-day-old cultures of two different strains of *C. immitis* growing on Sabouraud's medium. The average diameter of the zone of inhibition in the six cultures was 4.5 cm. The acute toxicity study performed in young mice averaging 21 g in weight gave the results shown in Table 1.

The extract has since been tried against *Microsporum gypseum* and a species of Trichophyton with moderate zones of inhibition; it is being tested against other fungi.

This green fungus has been tentatively identified by Chester Emmons at the National Institutes of Health as *Penicillium janthinellum* (*1*).

MARGARET C. EGEBERG
ANN F. ELCONIN
ROGER O. EGEBERG
Los Angeles County Hospital and
University of Southern California
School of Medicine, Los Angeles

Note

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6 April 1961

Significance of Some Fossil Wood from California

In June 1952, N. L. Taliaferro, R. Taylor, and I found fragments of wood and gastropod shells in a sandstone boulder 600 feet above sea level in Angus Canyon, a tributary to Capay Valley, about 40 miles west-northwest of Sacramento, Calif. The wood was later identified (by L. H. Daugherty) as *Cupressinoxylon*, and the gastropods (by J. W. Durham) as *Cophocara*.

Although the boulder is firmly embedded in a conglomerate near the base of the Eocene Capay formation, it is dated as upper Cretaceous by its gastropods and by lithologic comparison with nearby upper Cretaceous sandstone exposures. Analyses were made on parts of the dark-brown wood fragment shown in Fig. 1.

The specific gravity, determined by immersing fine splinters of the wood in heavy liquids, ranges from about 2.3 to 2.7. X-ray analysis of a splinter (by Adolf Pabst) showed it to be chiefly calcite. Ignition of a small piece showed the ash content to be 51.6 percent by weight. This ash was unaffected by an Alnico magnet, suggesting that no magnetic iron was present, and hence that no pyrite was present in the original unburned sample (*1*). The ash, x-rayed by Pabst, was found to be calcium oxide with a trace of hydrated calcium oxide.

If, as the x-ray analysis suggested, the ash was wholly calcium oxide, calcined from calcite, one can easily calculate the weight percentage of calcite in the original—namely, 92.1 percent. This explains, in part, the high specific gravity of the wood.

A transverse thin section (Fig. 2) shows that the original vegetal structure is excellently preserved. Evidently the calcite permineralization occurred before decomposition had progressed far enough to weaken the cell walls. Indeed, in view of Goldberg and Parker's reconstruction of phosphatization of wood (*2*), it appears likely that calcification was synchronous with oxidation of the tissue.

With crossed nicols, both transverse and tangential thin sections show that the lumina are filled with calcite, but opaque cell walls make up almost half the apparent volume. In view of the high specific gravity, the cell walls, as well as the lumina, must be impregnated with calcite.

Transverse sections also show that much of the calcite is fine-granular, unlike that in the calcified wood described by Greenland and Wherry (*3*); individual grains are generally smaller than the lumina they fill, so that many cells contain more than one individual. C-axes of several of these, measured on a Universal stage, appear to be randomly oriented. The relatively fine granularity of the calcite suggests that crystallization began at closely spaced centers and proceeded rather uniformly and rapidly. This suggestion supports the inference that permineralization was synchronous with initial decomposition, presumably soon after deposition. Also supporting this idea is the fact that the wood, both micro- and macroscopically, appears to have been but little deformed and flattened by the weight of overlying sediments before calcification.

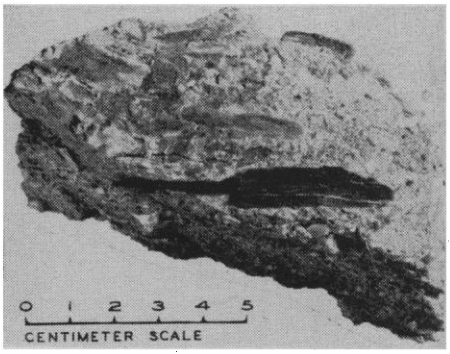

Fig. 1. Rock fragment showing piece of dark calcified *Cupressinoxylon* wood. Streaky gray fragments are other calcified wood chips; white fragments are shells of *Cophocara*, a gastropod with upper Cretaceous affinities.

The presence of *Cophocara* shells shows that the wood was deposited in a marine environment; coarse granularity of the sandstone suggests relatively strong local currents; absence of pyrite suggests relatively free circulation of the water. All of this points to an environment unlike that in which the wood of the Carboniferous coal balls was calcified—a restricted, anaerobic, lagoon or flood-plain environment.

The environment, though marine, was also different from that of the 410-m terrace, in the Gulf of Tehuantepec, from which Goldberg and Parker dredged phosphatized wood (*2*). They and others have suggested that, since the hydrogen-ion concentration requirements are similar for precipitation of calcium carbonate and calcium phosphate, the relative concentration of carbonate and phosphate ions in the water will determine whether calcite or apatite will be deposited.

Presumably, then, if the phosphate content of the Central Valley Cretaceous geosyncline was relatively low,

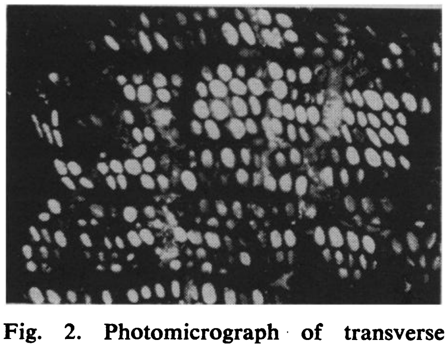

Fig. 2. Photomicrograph of transverse section of the wood fragment shown in Fig. 1. Crossed nicols. Width of view is approximately 0.45 mm.

calcite rather than apatite would have been deposited. The supposition that the phosphate content of the water was low is supported by the general paucity of Cretaceous fossil remains, especially of planktonic Foraminifera, in central California. A thriving marine animal community requires waters rich in phosphate. Such waters generally result from upwelling along open coasts. Hence, if the sea in the central California Cretaceous geosyncline was deficient in phosphate, as suggested by wood calcification and scarce marine fossils, it very likely was not receiving upwelled coastal marine waters. This line of reasoning tends to confirm the view of some geologists that the geosyncline was at least partially blocked off from the open sea during much of its existence.

CHARLES G. HIGGINS
Department of Geological Sciences, University of California, Davis

References and Notes

1. Ignition, under identical conditions, of black, Recent wood from another locality yielded ash that was strongly magnetic. The source of the magnetic iron is presumed to be pyrite in the original sample.
→ E. D. Goldberg and R. H. Parker, *Bull. Geol. Soc. Amer.* **71**, 631 (1960).
3. C. W. Greenland, *Econ. Geol.* **13** (1918); E. T. Wherry, *Proc. U.S. Natl. Museum* **53** (1917).

17 April 1961

Benzpyrenes in Soil

Abstract. Benzpyrenes appear to be common and fairly abundant constituents of soils. Both the carcinogenic 3,4-benzpyrene and its inactive 1,2-isomer have been detected spectroscopically in extracts of soils from rural areas of the eastern United States.

Polynuclear aromatic hydrocarbons are commonly found in recent marine and nonmarine sediments (*1*, *2*). Evidence has now been obtained that both the strongly carcinogenic hydrocarbon 3,4-benzpyrene and its inactive isomer 1,2-benzpyrene are common and fairby abundant constituents of solids.

Table 1. 3,4-Benzpyrene in soils.

Origin and type of soil	Conc. (μg/kg)
Oak forest, West Falmouth, Cape Cod, Mass.	40
Pine Forest, West Falmouth, Cape Cod, Mass.	40
Mixed forest, West Falmouth, Cape Cod, Mass.	1300
Mixed forest, eastern Connecticut	240
Garden soil, West Falmouth, Cape Cod, Mass.	90
Plowed field, eastern Connecticut	900

A number of soils from rural areas of Massachusetts and Connecticut were analyzed. After the samples were dried to constant weight at 110°C, 50 g of each soil sample were weighed and extracted in a Soxhlet by benzene-methanol (1:1). The solvent was then removed in a rotating evaporator, and the remaining extract was treated with warm iso-octane-benzene (3:2). The soluble fraction was again dried, taken up in boiling iso-octane, and immediately adsorbed on an 8-ml column of alumina (Brockman II). The elution was carried out with a graded series of iso-octane-benzene mixtures. In some cases, yellow nonhydrocarbon materials broke through in the hydrocarbon-fractions; they were then removed by rechromatography under identical conditions. The hydrocarbons in the eluates were detected by ultraviolet spectrophometry (Cary model 14 spectrophotomer), with a Sawicki chart (*3*) and a punched-card file of hydrocarbon spectra as aids in their identification. The spectra of 3,4-benzpyrene and 1,12-benzperylene, which both occur in soils, are very similar, but the presence of fine structure in the 383-mμ band together with a high 403-mμ band constitute conclusive evidence for the presence of 3,4-benzpyrene (*3*).

The hydrocarbon assemblage in all soil samples was found to be very similar even if the concentrations varied. The 1,2- and 3,4- isomers of benzpyrene were detected in all samples. Figure 1 is the spectrum of a typical benzpyrene fraction, contaminated with some chrysene (responsible for the background absorption) and some perylene (peaks at 434 and 428 mμ). The concentration of the biochemically active 3,4-benzpyrene was estimated from the intensity of the 403-mμ band (Table 1). In addition to the benzpyrenes, the following hydrocarbons were represented in all samples: phenanthrene, fluoranthene, pyrene, chrysene, perylene, and anthanthrene. Extensive rechromatography of the combined extracts provided evidence for the additional presence of anthracene, triphenylene, benzanthracene, benzfluorene, 1,12-benzperylene, and coronene.

Some soils may contain a much higher concentration of 3,4-benzpyrene than reported here. Kern (*1*), in his paper on the discovery of chrysene in some Swiss soils, describes the isolation of two additional hydrocarbons, not identified with certainty. The reported spectra and analytical data lead us to conclude that Kern already had isolated 3,4-benzpyrene [melting point: 171°C (benzpyrene: 177°C); molecular weight: 283 (benzpyrene: 252); log $\epsilon^{1\ \mathrm{percent}}_{1\ \mathrm{cm}}$ (at 296 mμ): 1.7×10^3 (benzpyrene: 2.2×10^3)]. According to Kern, this hydrocarbon was obtained in crystalline form with the remarkable yield of 21 mg/kg of soil.

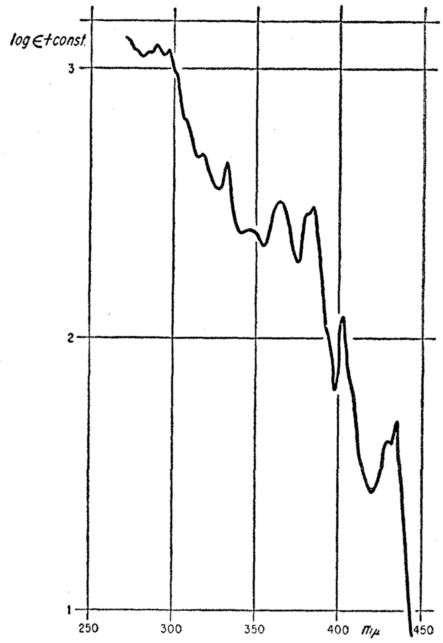

Fig. 1. Spectrum of a typical benzpyrene fraction, contaminated with some chrysene and some perylene (in iso-octane).

We believe that the occurrence of such hydrocarbon concentrations in rural soils distant from major highways and industries cannot be ascribed to fallout from polluted air. More likely, these hydrocarbons are indigenous to soil. They are among the pyrolytic products of wood and might be formed in soil by related low-temperature processes, as they also occur in the transformation of plant organic matter to peat and lignite. Alternatively, the hydrocarbons might be the products of the organisms which contribute their organic matter to the soils. These genetic mechanisms—if correct—imply that man has been in contact with carcinogenic hydrocarbons, not only during the industrial epoch, but during his entire history. It remains to be examined whether the concentration and availability of the benzpyrene in soil is sufficient to be of concern to those exposed to continued contact with soil (*4*).

MAX BLUMER
Woods Hole Oceanographic Institution, Woods Hole, Massachusetts