

evidence. On the other hand, Venus observations in this wavelength range and at other wavelengths are entirely compatible with the reflection spectrum of a noninfinite cloud layer composed of very small or slender ice particles (12).

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5 December 1968

Triterpene Alcohol Isolation from Oil Shale

Abstract. *Isoarborinol, an intact pentacyclic unsaturated alcohol, was isolated from the Messel oil shale (about 50×10^6 years old). Complex organic substances, even those very sensitive to oxidation, reduction, or acidic conditions, can thus survive without alteration for long periods.*

From the Eocene oil shale of the Messel mine near Darmstadt, Germany, we have isolated an intact triterpene alcohol, isoarborinol (Fig. 1). This nonmarine oil shale has never been buried deeper than 200 to 300 meters and is thus a typical "unripe" sediment (1).

The samples of shale were finely layered and contained about 30 percent organic carbon. After being dried in a vacuum, shale (1000 g) was crushed to 2-cm pieces and cleaned with a mixture of benzene and methanol (3:1 by volume). It was then pulverized and extracted with a mixture of petroleum ether and ethyl acetate (4:1 by volume) by ultrasonic vibration (25 khz). The extraction was repeated twice, with 100 ml for 50 g of rock. Decantation, centrifugation, and evaporation of the solvents gave 9.9 g of residue, which

was then chromatographed on Merck silica gel (0.05 to 0.2 mm). Three fractions were eluted—F₁ with petroleum ether, F₂ (4.7 g) with petroleum ether and ethyl acetate (4:1 by volume), and F₃ with benzene and methanol (3:1 by volume). Fraction F₂ was again chromatographed over silica gel and gave 300 mg of a crystalline mixture of secondary alcohols (infrared spectrum) of the same polarity as triterpene alcohols. They were acetylated (pyridine, acetic anhydride) and again chromatographed over silica gel impregnated with 10 percent silver nitrate (elution with cyclohexane and benzene, 3:1 by volume). One of the fractions (80 mg) proved homogeneous upon gas chromatography (2) and could be identified with isoarborinol acetate (Fig. 1) by the following criteria: melting point, R_f on thin-layer chromatography over silica gel and 10 percent silver nitrate, gas chromatography, and infrared and mass spectrometry.

Isoarborinol had never been isolated in our laboratory, and control experiments excluded the possibility of contamination.

The group of arborane triterpenoids is relatively new. Isoarborinol has been isolated from *Glycosmis arborea* (Rutaceae) (3), *Hedyotis acutangula* (Rubiaceae) (4), and *Imperata cylindrica* var. *koenigii* (Gramineae) (5). Its

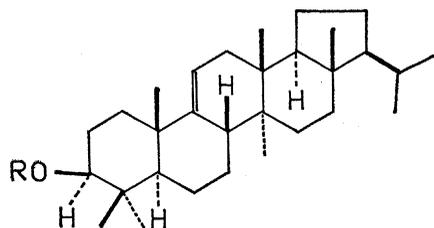


Fig. 1. Triterpene alcohol isolated from oil shale. R = H, isoarborinol. R = CH₃CO₂-, isoarborinol acetate.

structure was defined by x-ray crystallography (6).

Triterpenoids have frequently been found in petroleum, in coal, or in sediments (7). It is, however, only from relatively young brown coal that intact triterpenes have been isolated (8). In the other cases, "aging" has been brought about by the acidic, oxidizing, reducing, or thermal conditions prevailing in the rock, and the substances isolated are modified derivatives of extant triterpenes (9).

The fact that isoarborinol is sensitive to oxidation, reduction, and acid treatment requires that mild conditions have prevailed during diagenesis in the Messel mine (10). In contrast to recent cases reported from our laboratory (11), we do not know from which of the fossil plants of the shale isoarborinol originates, and we must refrain from any comments on this point until several companions of this triterpene have been characterized. It is, however, remarkable that isoarborinol has so far been isolated only from tropical plants, and that the fossil flora and fauna found in the Messel oil shale are near relatives of the recent flora and fauna of South East Asia (12).

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13. Supported by DGRST grant 64-FR-058. We thank the Ytong AG and the management of the Messel mine for shale samples; J. Lucas, C. Sittler, and H. Knoche for discussion and general help; B. van der Weide for determination of organic carbon content; P. Witz and G. Teller for mass spectra, and S. Natori for authentic comparison sample of isoarborinol acetate.

20 September 1968; revised 30 December 1968 ■

Subacute Sclerosing Panencephalitis: Propagation of Measles Virus from Brain Biopsy in Tissue Culture

Abstract. *Measles virus was propagated in monolayer cultures established from brain tissue of a patient with subacute sclerosing panencephalitis. Syncytial cells were rendered fluorescent with measles specific antisera only, by means of an indirect technique. The ultrastructural appearance of the microtubular aggregates was identical in brain tissue and in the cultured cells. Fusion experiments produced a cytopathic effect in human embryonic kidney and VERO cell cultures. The virus was identified by hemagglutination-inhibition, but only in the supernatant of disrupted cultured cells.*

Subacute sclerosing panencephalitis (SSPE) is a disease of children and adolescents. Intellectual deterioration or convulsive disorders are the usual presenting signs followed by motor disturbances of the extrapyramidal type and myoclonic jerks. The electroencephalogram presents synchronous, slow-frequency, high-voltage waves. Spinal fluid and serum contain increased amounts of immunoglobulin G, frequently associated with M-gradients and with abnormalities in the precipitation arc of gamma globulin (1). Although Dawson suspected a viral etiology as early as 1933 (2), it was not until 1965 that measles virus was incriminated on the basis of electron microscopic observations (3). This assumption has been supported by immunological findings: high levels of measles antibodies in serum, spinal fluid, and brain tissue extracts (4); and demonstration of intracellular measles antigen with fluorescein-labeled measles antibody (5).

Two groups of investigators succeeded in transmitting an encephalogenic agent from brains of patients to experimental animals, but the agent was not identified as measles virus (6). Recently, propagation of measles virus has been achieved in tissue culture (7). We now confirm and extend these observations.

The patient, an 8-year-old white boy, exhibited the characteristic evolution and clinical picture of SSPE, except that he allegedly had neither been ex-

posed to measles nor vaccinated against the virus. He had serum titers of measles antibodies of 1:512 for hemagglutination-inhibition and 1:64 for complement-fixation. The patient developed delayed hypersensitivity to homologous skin grafts and to dinitrochlorobenzene, but no delayed response was observed after intradermal injec-

tions of live and of killed measles virus (8).

A sample of brain tissue was obtained through a right frontal craniotomy on 4 October 1968. The brain appeared grossly normal, but histologic examination revealed the diagnostic findings of SSPE: intranuclear inclusion bodies of Cowdry type A, lymphoplasmocytic perivascular and interstitial infiltration, and microglial proliferation. Staining of acetone-fixed frozen sections with fluorescein-labeled rabbit antiserum to human gamma globulin (9) produced fluorescent staining of perivascular and interstitial mononuclear cells. No immunofluorescence studies were done on the biopsy tissue. Intranuclear and intracytoplasmic aggregates of microtubules, having a diameter of 20 to 22 nm, were identified by electron microscopy (Fig. 1).

A piece of brain tissue measuring 0.8 by 0.5 by 0.4 cm, including cortex and white matter, was washed four times in Puck's saline A (10) and minced into small pieces. Tissue fragments were incubated in 0.25 percent trypsin (11) at room temperature and stirred slowly. After 15 minutes the supernatant was transferred to a test tube containing an equal amount of culture medium NCTC 109 with 20 percent fetal calf serum (10). Fresh

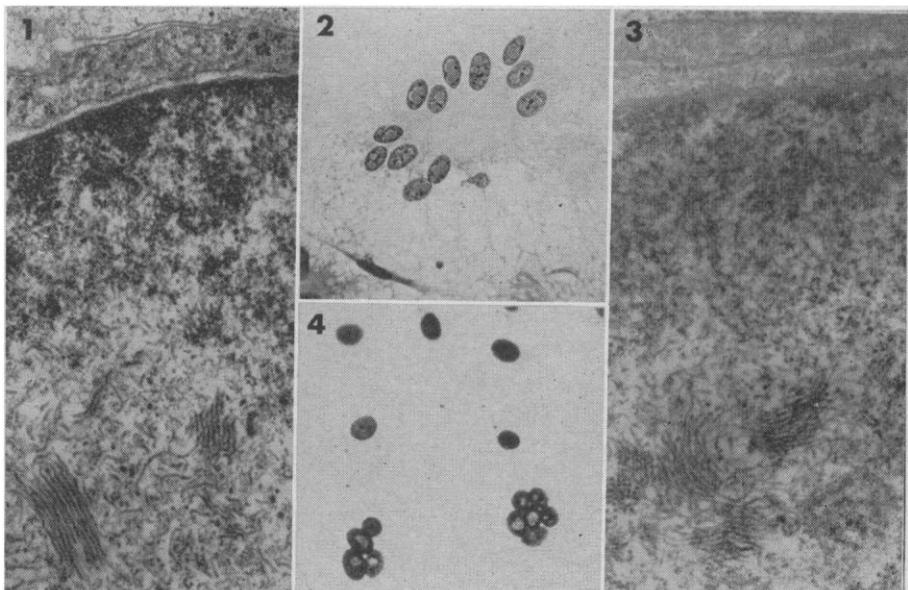


Fig. 1. Electron micrograph of brain biopsy showing oligodendrocytic nucleus with microtubules representing the ultrastructural correlate of the Cowdry type A inclusion body ($\times 18,100$). Fig. 2. Syncytial giant cell in secondary culture of brain cells from biopsy specimen showing eosinophilic intranuclear inclusion bodies (hematoxylin and eosin stain; $\times 240$). Fig. 3. Electron micrograph of syncytial giant cell from secondary culture of brain cells showing intranuclear aggregates of microtubules ($\times 21,300$). Fig. 4. Two syncytial giant cells with intranuclear inclusion bodies of Cowdry type A. VERO cell culture inoculated with infected brain cells (Giemsa stain; $\times 160$).