have curbed photosynthetic activity of green plants over large parts of the earth, resulting in a slight lowering of the oxygen content of the air. Such a hypothesis can be verified only by better determinations of the diffusibility of gases through ice and by more accurate laboratory procedures for the extraction and analysis of the gas. No particulate matter could be detected in our ice pieces, but we cannot exclude the possibility of oxygen loss from dust oxidation. Whether or not enough organic material can be obtained from the ice for radiocarbon dating we do not know.

In our random investigation we did not encounter ice with gas bubbles rich in oxygen, which is suggestive of frozen, air-saturated water such as one might encounter in temperate-type glacier ice.

The bubbles in our icebergs were found to be under pressure, usually between 2 and 6 atm. Why the pressure seems to vary so much from one part of a berg to another is a puzzling problem. If such pressure gradients can persist in the bergs for a long time they might reflect something of the history of the berg such as the depth in the glacier from which it came.

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3. Contribution No. 818 from the Woods Hole Oceanographic Institution. This work was carried out on the *Blue Dolphin* Labrador Expedition, 1954, under the auspices of the Arctic Institute of North America, project ONR-138, with funds provided by the Office of Naval Research.
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Synthesis of Coffinite—$USiO_4$

A recent communication by Stieff, Stern, and Sherwood (*1*) gave a preliminary description of a new mineral, coffinite ($USiO_4$), which has become recognized as a major uranium mineral on the Colorado Plateau. Coffinite is described as a fine-grained black mineral; it is best identified by its x-ray powder pattern. The crystal structure is tetragonal, isomorphous with thorite ($ThSiO_4$). It was also noted that all attempts to synthesize $USiO_4$ had been unsuccessful to date. We have spent some time on attempts to prepare $USiO_4$, and our work has now progressed to the point where a preliminary report can be made describing the synthesis of coffinite by a hydrothermal process (*2*). The synthetic coffinite has been identified by its crystal structure, even though pure material suitable for chemical analysis has not been isolated.

Our procedure is, briefly, as follows: 1 mmole each of uranium tetrachloride and sodium metasilicate are dissolved in 10 ml of water. Sodium hydroxide solution is added dropwise to the uranium-silicate solution until a stiff gel forms near the neutral point. Enough additional base is added to make the mixture slightly alkaline (*p*H 8 to 10) (*3*). The gelatinous precipitate is then centrifuged and transferred to a vitreous silica tube, which is placed in an Inconel bomb tube. We carry out these operations in a nitrogen atmosphere to prevent oxidation of the uranium. Whether this precaution is necessary remains to be determined. Other variables in the procedure must also be evaluated. The sealed Inconel tube is heated 4 to 5 days at 250°C to crystallize the $USiO_4$.

Synthetic coffinite appears as a bluish-green solid in the reaction products. The material prepared to date appears to be isotropic under the microscope, but x-ray powder patterns confirm the presence of tetragonal crystals of coffinite. Quartz or cristobalite have been identified as contaminants in all the coffinite preparations that have been made to date. A comparison of lattice dimensions of natural coffinites with the synthetic material gives the following: Arrowhead Mine (*1*), Mesa County, Colo., $a = 6.93$ kx, $c = 6.30$ kx; Jack Pile Mine (*4*), Laguna, N. M. (AE1019), $a = 6.937$ kx, $c = 6.285$ kx; and synthetic coffinite (*5*) $a = 6.977$ kx, $c = 6.307$ kx. The refractive index of synthetic coffinite has been found to be 1.83 to 1.85.

We have heated the black, naturally occurring coffinite (AE1019) in air at 375°C to oxidize away the organic matter with which it is associated. The inorganic residue retains the tetragonal structure, but is then a gray-green color that is characteristic of tetravalent uranium compounds and quite similar to that of the synthetic coffinite.

Naturally occurring $USiO_4$ is reported (*6*) to decompose to UO_2 and amorphous silica when it is heated above 400°C, although Grüner (*7*) reports retention of the tetragonal structure of the Jack Pile Mine coffinite on ignition to 500°C. Our synthetic coffinite has been found to be thermally stable, in vacuum, for at least 5 hours at 700°C.

Work on coffinite, its preparation and its properties, is continuing and will be reported in more detail at a later date.

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2. This work was performed under the auspices of the U.S. Atomic Energy Commission.
3. Our reaction products have invariably been UO_2 and silica whenever the mixture is allowed to remain slightly acidic during the heating process.
4. This sample was obtained through the courtesy of J. W. Grüner of the University of Minnesota.
5. Cell constants of the Jack Pile Mine and synthetic coffinite were measured by S. Siegel.
6. L. R. Stieff, personal communication.
7. J. W. Grüner, personal communication.

22 August 1955

Interference in Salkowski Assay of Indoleacetic Acid

The Salkowski reaction (*1*) has long been used as a simple assay for indole derivatives (*2*), although it is not entirely specific for them. In their study of the enzymatic destruction of growth substances, Tang and Bonner (*3*) utilized the Salkowski reaction as an assay for indoleacetic acid (IAA), one of the few compounds that gives a carmine-pink color with the reagent (*2*). Lately the reaction, especially in the modification of Gordon and Weber (*4*), has been widely used for the assay of IAA and other indole-containing growth substances, both in solution and on paper chromatograms. The colored product has recently been ascribed to hydroxylation of the indole nitrogen (*5*).

The assay method is, however, subject to interference from various sources. Siegel and Weintraub (*6*) and others have noted that peroxides interfere by making the pink color too fugitive to be measured, and Brauner (*7*) observed that the reaction is inhibited by light. The experiments described in this report show that a number of other commonly occurring compounds may regularly interfere with the assay and that important changes are caused by exposure to light during, before, or after color development (*8*).

Curve *A* in Fig. 1 shows the usual time course of the development of the pink color when the reagent of Gordon and Weber (*4*) is used. The sample, before reagent was added, contained $4 \times 10^{-5}M$ IAA (*9*). A stable maximum in curve *A* is reached in less than 30 minutes. If, however, the reagent has been exposed to bright light beforehand, the color development is greatly delayed (curve *B* in Fig. 1). Illumination of the reagent made up with sulfuric acid (*3*) also gives a color delay. Furthermore, a similar delay results if a ferrous salt is added to the reagent. Curve *B* duplicates almost exactly that produced by using a perchloric acid Salkowski reagent in which 7 percent of the prescribed iron is in the ferrous state. It seems probable that the effect of light results from the formation of a small amount of ferrous ion. The observation that x-rays can reduce ferric to ferrous ion even in the presence of perchloric acid (*10*) is a precedent for this interpretation.

It might be thought that, with care to keep the complete reagent in a minimum of light, ferrous interference could easily be dismissed from routine Salkowski assays. But, unfortunately, such interference may also be observed when the assayed sample contains reductants such as hydroquinone, ascorbic acid, or cysteine. Ascorbic acid, for example, at $4 \times 10^{-4}M$ produces a color delay effect that would correspond to an almost stoichiometric reduction of the iron in the reagent.

Inasmuch as the color development

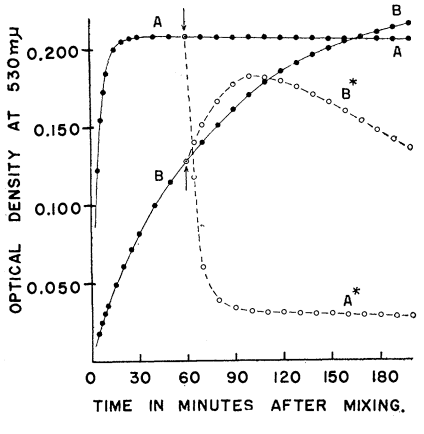

Fig. 1. Time course of Salkowski color development. (Curve A) "Normal" development, method of Gordon and Weber (*4*), $4 \times 10^{-5}M$ IAA in sample; (curve B) same as curve *A* but with Gordon and Weber reagent exposed to about 500 ft-ca of white light (fluorescent tube) before use; (curves *A** and *B**) arrows indicate time of start of exposure of reaction mixtures *A* and *B*, respectively, to white light.

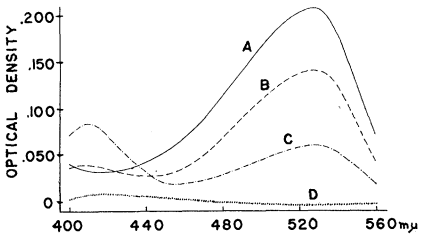

Fig. 2. Absorption spectra of samples after one hour's reaction with Salkowski reagent, measured against a water-diluted Salkowski reagent blank. (*A*) $4 \times 10^{-5}M$ IAA; (*B*) $4 \times 10^{-5}M$ IAA and $4 \times 10^{-5}M$ catechol; (*C*) $4 \times 10^{-5}M$ IAA and $2 \times 10^{-4}M$ catechol; (*D*) $2 \times 10^{-4}M$ catechol.

may be delayed by Fe^{++} and other reductants and accelerated by H_2O_2, it seems probable that the normal color is due to a slow oxidation. For assays of IAA alone, the unmodified color development is usually satisfactory, but indoleacetonitrile, for example, reacts very slowly with the standard reagent. The addition of a small amount of H_2O_2 (about $3.10^{-5}M$) to the mixture hastens color development and somewhat improves the maximum intensity reached with this compound.

It is notable that the rapidly formed ferric-IAA color (curve *A* of Fig. 1) is much more sensitive to bleaching than the color formed in the presence of ferrous ion (curve *B*). After 1 hour of dark development, replicate mixtures were exposed to the light of one fluorescent tube (about 500 ft-ca). The subsequent density changes in the "normal" are shown in curve *A**; within 10 minutes, the color has been bleached to less than a third of the maximum developed in the dark. The ferrous-delayed color reacts as shown in curve *B** when it is exposed to the same light. The color development is slightly accelerated for about 40 minutes, after which a slow bleaching ensues.

Another type of interference with the Salkowski reaction has been observed in dealing with plant extracts. Curve *A* in Fig. 2 shows parts of the absorption spectrum of the colored product that was formed when the perchloric reagent (*4*) was used on a sample containing $4 \times 10^{-5}M$ IAA. If such a sample contains, in addition to IAA, an equimolar concentration of catechol, as in curve *B* of Fig. 2, there is no delay in the color development, but the final density of the reaction mixture at 530 mμ is decreased by about 30 percent. Curve *C* shows the absorption spectrum 1 hour after Salkowski reagent was mixed with a solution containing both $4 \times 10^{-5}M$ IAA and $2 \times 10^{-4}M$ catechol. When this concentration of catechol alone has reacted with the reagent for 1 hour (curve *D*), it is hardly detectable by any absorption in the given range; but when it is included in the sample with IAA, it diminishes by more than 70 percent the absorption at 530 mμ that is expected from the ferric-IAA reaction. It is also evident in Fig. 2, curve *C*, that the ferric-IAA-catechol mixture develops an absorption peak near 410 mμ even at these low concentrations of catechol. This suggests that the ferric ion in this case is catalyzing an additional reaction in which both IAA and catechol participate.

Other polyphenols such as resorcinol, phloroglucinol, and hydroquinone also inhibit the development of the pink ferric-IAA absorption, but the interference by hydroquinone and some other phenols (for example, catechin) is particularly complicated, perhaps through combining the catechol type of interference with a simultaneous reduction of Fe^{+++}.

Reducing agents and polyphenols are so widespread in plants, and so drastic in their Salkowski interference, that the Salkowski assay, either in solution or on chromatograms, should be considered uncertain when one is dealing with any but the most highly purified of plant extracts. On the other hand, the recognition of these effects on the test may well suggest other important characteristics of the assayed material (*11*).

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