types of pulverulent uraninite from Shinkolobwe suggest a high UO_3 content for this material. At Marysvale and Caribou hard, brittle uraninite has been found in association with the sooty material, the latter coating and penetrating the solid brittle ore.

Although the sooty mineral is later, it is found at such depths that it apparently does not represent a typical surface-weathering product. At White Canyon it is found 50–100 ft underground from the tunnel portal but not directly at the surface. At Marysvale, it is found a corresponding depth below the surface and, in addition, in lower mine workings in the Prospector mine. At Caribou the sooty mineral occurs at the 1,040-ft level and below. At Bellvue-Rochester it lies far below the surface.

The environment in which the sooty mineral is found is more or less porous and permeable to solutions. At the two Colorado localities and at Marysvale, it occurs in an envelope of fractured clay, having a thickness of several feet on either side of the uranium-bearing core where the enclosing wall rock has been altered to clay and associated porous minerals. At White Canyon the wall rock is a more or less horizontal stratum of porous sandstone about 10 ft thick, with shale above and below. The porous character of the environment in which the black powder is found, even including the associated host rock on the Shinkolobwe specimens, indicates an enclosing zone permeable to solutions. Observations at the surface itself and a few feet below the outcrop are inconsistent with the assumption that the sooty mineral is the result of simple weathering, since the pulverulent material, if formed in such a way, should persist to the surface. In the outcrop zone at Marysvale and White Canyon, yellow or green oxidized uranium minerals, typical of surface weathering, occur in place of the sooty material.

Associated metallic sulfides of copper at White Canyon, and zinc and lead as well at Caribou, point to the likelihood that the originating temperatures were higher than would prevail for normal ground water. Indicators belonging to the temperature scale of the geologic thermometer have been observed in the form of vein fluorite, chalcopyrite, and sphalerite, but the temperatures of formation are indefinite.

While studies are still in progress and further publication is in preparation, it seems reasonable to conclude that solutions and complete submersion, rather than simple weathering and surface oxidation, must be assumed responsible for the sooty uraninite. Since the mineral is prevailingly uraninite and occurs in a hydrothermal environment, it also seems reasonable to suggest that the solutions from which precipitation occurred were heated.

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Plant Growth-regulating Activity in Certain Aryloxyalkylcarboxylic Acids

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In our first report on substitution into the side chain of certain aryloxyacetic acids (*1*), it was suggested that the presence of a hydrogen atom on the carbon adjacent to the carboxyl group may be necessary for certain types of plant growth-regulating activity. Further compounds have now been synthesized, the assessment of biological activity has been extended by the inclusion of other tests, and additional evidence obtained to support our original suggestion. The following classes have been investigated, in which the aryl groups are phenyl, 2-chlorophenyl, 4-chlorophenyl, 2 : 4-dichlorophenyl, 2-methyl-4-chlorophenyl, 2 : 4 : 5-trichlorophenyl, 1-naphthyl, and 2-naphthyl:

$ArOCH_2COOH$ (I) aryloxyacetic acid	$ArOCH(CH_3)COOH$ (II) α-(aryloxy)-propionic acid
$ArOCH(C_2H_5)COOH$ (III) α-(aryloxy)-*n*-butyric acid	$ArOC(CH_3)_2COOH$ (IV) α-(aryloxy)-*iso*butyric acid

It was previously shown (*1*) that four aryloxy*iso*butyric acids failed to induce responses in the tomato leaf epinasty test (*2*). These findings have been confirmed, all the eight *iso*butyric acids listed above being inactive in this test, whereas with the exception of phenoxyacetic acid, all the acetic, propionic, and *n*-butyric acids (I, II, and III) were active.

Other methods we have employed for assessing growth-regulating activity have included the *Avena* curvature (*3*), *Avena* cylinder (*4*), tomato parthenocarpy (*5*), tomato leaf rooting (*6*), and Went pea curvature (*7*) tests. In addition, the capacity to induce morphological changes in the growth of tomato plants has been studied (*8*).

In general, it was found that compounds that possess at least one hydrogen attached to the α-carbon of the side chain (I, II, and III) give a positive response in the tomato leaf epinasty, *Avena* curvature, and *Avena* cylinder tests, all of which depend upon cell elongation. Compounds in which such hydrogen atoms had been substituted by methyl groups (IV) were found to be uniformly inactive in these tests at the concentrations employed; indeed, certain of the compounds appeared to inhibit the normal growth of cells.

In tests involving cell division—e.g., tomato parthenocarpy, leaf rooting, and production of morphological effects—most of the *iso*butyric acids (IV) were inactive. However, 2 : 4-dichloro-, 2-methyl-4-chloro-, and 2 : 4 : 5-trichlorophenoxy*iso*butyric acids showed

some, though in all cases a lower, activity than the corresponding acetic, propionic, and *n*-butyric acids.

Within the range of acids tested, all acetic, propionic, and *n*-butyric derivatives (I, II, III) with the exception of phenoxyacetic acid, induced positive curvatures in the pea test. Some activity was also shown by certain *iso*butyric acids (IV) in this test, though phenoxy-, 2-chloro-, and 4-chlorophenoxy*iso*-butyric acids were inactive. The pea test, however, cannot be classified as assessing simple cell extension or cell division (*8*). A more detailed study of all these compounds in the pea test is now the subject of a separate investigation.

Our results indicate that, in general, the α-(aryloxy)-*iso*butyric acid structure is not associated with high growth-regulating activity. In particular, such compounds do not stimulate cell extension in the test methods we have employed.

Further details of this work will be published elsewhere.

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Crystalline α-Lipoic Acid: A Catalytic Agent Associated with Pyruvate Dehydrogenase

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A crystalline compound which in catalytic amounts can replace the growth-stimulating activity of acetate for certain lactic acid bacteria, and which is required for the oxidative decarboxylation of pyruvate by these bacteria, has been prepared from liver.

The stimulatory effect of acetate on growth of lactic acid bacteria was first demonstrated by Snell *et al.* in 1937 (*1*). Guirard *et al.* (*2*) in 1946 reported that some biological preparations contain substance(s) which replace acetate as a growth stimulant for several lactic acid bacteria. The concentration of the acetate-replacing factor(s) was undertaken by these investigators, continued by Getzendaner (*3*), and then by one of the present authors (LJR) and his collaborators.

In 1947 O'Kane and Gunsalus reported (*4*) that an unidentified factor, designated pyruvate oxidase factor (*5*), is required for pyruvate oxidation and dismutation by *Streptococcus faecalis*. Further details of their work were presented at the Gordon Research Conferences at New London in 1949.

TABLE 1

X-RAY CHARACTERIZATION* OF CRYSTALLINE α-LIPOIC ACID

"*d*" Interplanar spacings	I/I° Relative intensity
5.30	.10
4.82	1.00
4.52	.10
4.24	.10
4.04	.80
3.85	.50
3.53	.10
2.99	.10

* The data were obtained on a 114-ml Norelco powder camera using Cu Kα radiation. The sample was mounted in a Parlodion capillary. Those lines possessing less than 10% of the intensity of the strongest band have been omitted.

In 1949 Snell and Broquist reported (*6*) that concentrates of the pyruvate oxidase factor and of "protogen," an essential growth factor for *Tetrahymena geleii* described by Stokstad *et al.* (*7*) were very active in promoting growth of *Lactobacillus casei* in the absence of acetate.

In the fall of 1950 a collaborative program was undertaken by the present authors and Eli Lilly and Company.[1] This work has led to the obtaining of a crystalline compound from processed insoluble liver residues, which is highly active for the growth of *Streptococcus lactis* in the absence of acetate and as an activator of the apo-pyruvate dehydrogenase of *S. faecalis*. This compound is being called α-lipoic acid.[2] The potency of crystalline α-lipoic acid is about 250,000 pyruvate oxidase factor units/mg.[3] In the *S. lactis* assay it possesses about 15,000,000 acetate units/mg;[3] i.e., 1.7×10^{-6} μg/ml of culture medium is capable of supporting half-maximal growth in the absence of acetate.

α-Lipoic acid is very soluble in organic solvents, but only sparingly soluble in water. This compound, as obtained, crystallized in the form of platelets possessing a faint yellow tinge, which melted on the microstage at 47.5°–48.5°. It is an acidic substance possessing a pKa of 4.7. The x-ray diffraction data of the crystalline product are presented in Table 1.

[1] The authors are indebted to Eli Lilly and Company for supplying liver concentrates and for measuring the physical constants reported for the crystalline product.

[2] The name lipoic acid is derived from the fact that the compound is highly soluble in fat solvents, is acidic, and is involved, through oxidative decarboxylation of pyruvate, in the formation of acetate, a precursor of fatty acids. The crystalline compound reported in this paper is designated as α-lipoic acid to indicate that it is the first member to be obtained of a series of chemically related substances which possess acetate-replacing and pyruvate oxidase factor activity. The terms acetate-replacing factor and pyruvate oxidase factor were used previously to indicate biological activity and not to denote specific compounds.

[3] The details of these assays will be reported elsewhere. One pyruvate oxidase unit is equivalent to the manometric response produced by 1 mg yeast extract. One acetate unit is equivalent to the growth response produced by 1 mg sodium acetate.