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

Lester J. Reed, Betty G. DeBusk, I. C. Gunsalus, and Carl S. Hornberger, Jr.

Biochemical Institute* and *Department of Chemistry*, *University of Texas*, and *Clayton Foundation for Research, Austin, Texas;* and *Department of Bacteriology*, *University of Illinois, Urbana

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.

The fact that materials possessing the biological activity of α-lipoic acid can be recovered from diverse biological sources, coupled with the fact that α-lipoic acid has extremely high biological activity and possesses a catalytic role (in the oxidative decarboxylation of pyruvate), suggests that it is a new member of the family of B vitamins. Further research is in progress on its composition, structure, and biological activity.

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The Green Pigment and Physiology of Guard Cells

R. O. Freeland

Department of Botany,
Northwestern University, Evanston, Illinois

There is widespread uncertainty in the minds of botanists as to the composition and physiology of the green pigment located in plastids in the guard cells of most leafy plants. Sayre (*1*) concluded from his extensive study of the physiology of the stomates of *Rumex patientia* that the plastids in the guard cells are different structurally, physiologically, and genetically from the chloroplasts of the mesophyll cells. Using microchemical methods, he was unable to obtain a positive test for chlorophyll in the plastids of guard cells but stated that there is no conclusive proof that it is not chlorophyll, because of the extreme difficulty of making the test upon such small bodies.

The present paper deals with two problems related to guard cells, the determination of the absorption spectrum of the green pigments, and a test for photosynthesis in them.

After examining many plants, the leaves of *Hymenocallis littoralis,* Salisb., were found to be most suitable for investigation. In this species, large pieces of epidermis can be stripped from the leaves, and the presence of a heavy cuticle renders it relatively easy to smooth and straighten them for cleaning and inspection. The latter is important because freshly stripped pieces of epidermis were always found to have some fragments of mesophyll adhering to them. Each piece of epidermis, after being mounted on a glass plate, was scraped on the inner surface with a safety razor blade and then scrubbed with a soft bristle brush and water until it was free of all adhering cells and plastids from the mesophyll. Each strip of tissue was inspected under a microscope and if satisfactory was placed in a darkened test tube of cold acetone to which a pinch of $CaCO_3$ had been added. This procedure was continued at intervals over a period of a month or more until a 30-ml test tube full of loosely packed epidermal tissue was accumulated.

The plant material, plus a little quartz sand, was ground in the acetone with a mortar and pestle. After filtering, the acetone solution was light-green in color. The acetone extract was added to 50 ml of a petroleum ether-acetone mixture (10:1) in a separatory funnel. Gentle rotation and the addition of a small quantity of water brought about separation of the two solvent phases. The water-acetone layer was discarded, and the remaining petroleum ether was washed repeatedly with water. Then enough benzene was added to the petroleum ether to give a solution composed of 9 parts petroleum ether to 1 part benzene. This solution, containing the pigments, was passed, by gravity flow, through an adsorption column 1.5 cm in diameter, consisting from top to bottom of 20 cm powdered sugar, 5 cm $CaCO_3$, and 5 cm alumina. Finally, the column was treated with 10% benzene in petroleum ether in an attempt to resolve any possible components of the adsorbed pigment in a well-defined chromatogram. Only one pigment layer, blue-green in color, was visible in the column. After drying the column by suction with air, this layer was removed. The pigment was eluted from the sugar with a 1:1 solution of methanol and ethyl ether. The extract was freed of methanol by repeated water washing in a separatory funnel. The resulting ethyl ether containing the green pigment was studied immediately in a Coleman Junior spectrophotometer. The data on light absorption in terms of density are presented in Fig. 1. The entire experiment, from the collection of epidermal tissue to pigment analysis, has been repeated with the same qualitative results. For comparative purposes the pigments were extracted from the mesophyll tissues, separated, and studied in the same way. The chromatogram for the pigments from the mesophyll showed the usual distribution from top to bottom of the adsorption column of xanthophyll,

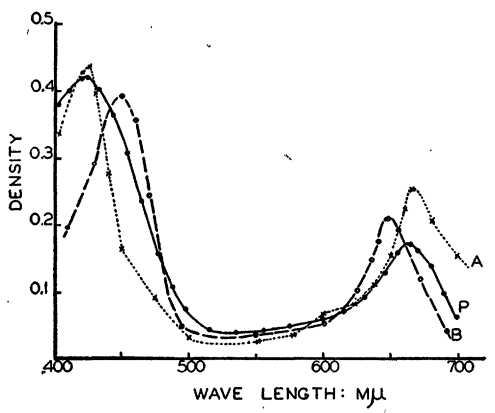

FIG. 1. Absorption data for ethyl ether solutions of the green pigments extracted from the leaves of *Hymenocallis. P,* pigment from the guard cells: *A* and *B*, chlorophylls *a* and *b*, respectively, from the mesophyll. (Density = − log Transmittance.)