

α -phosphonocinnamates, but that the *p*-nitro derivative is unstable and loses the phosphono group to give only ethyl *p*-nitrocinnamate as the product. W. S. Johnson later suggested that it was more likely that the reaction proceeded by a Wittig type elimination in the case of the *p*-nitro derivative.

We will present evidence that, in fact, both the phosphono ester and the dephosphonated ethyl cinnamates are obtained in most, if not all, of these reactions; that the reaction proceeds by both the Knoevenagel and the Wittig reactions simultaneously; and that the relative rates of these reactions are dependent on the electron withdrawing or donating ability of the substituent group.

An Examination of the Isomer Distribution in the Preparation of Ethyl α -Acetylcinnamates. WILLIAM DAVID BRYAN and CHARLES N. ROBINSON, Memphis State University.

The condensation of acetoacetic ester with substituted aromatic aldehydes has been known for nearly a century; however, the properties of the products which were reported indicated that only the *Z*-isomers were formed, though most authors have simply ignored the geometry of the products.

In our study of this reaction (using both *m*- and *p*-substituted aldehydes) we have found that both the *E* and *Z*-isomers are formed in all of these reactions, but that when the products are distilled and allowed to solidify slowly at room temperature the *Z*-isomer solidifies first and the remaining liquid continues to equilibrate until all of it is converted into the *Z*-isomer in the case of the *p*-substituted compounds. Most of the *m*-substituted do not become solids at room temperature and thus remain as equilibrium mixtures at room temperature.

"Window Cliffs" Natural Bridge Complex. STEPHEN NEELY & YASMIN MAIS, Austin Peay State University.

"Window Cliffs" natural bridge complex is located in southwestern Putnam County, Tennessee. The complex occurs in a ridge approximately 115 ft. above a meander in Cane Creek and about 2 kilometers northwest of Burgess Falls Natural Area.

The bridge complex occurs on a portion of the Eastern Highland Rim escarpment. Stratigraphically, the bridge complex is in the Ft. Payne formation. The bridge complex consists of four bridges of varying sizes. The largest bridge (bridge #1) has a span of 6.8 meters and its clearance is 4.5 meters high. Bridge #2, has a span of 2.3 meters, clearance 2.0 meters; Bridge #3,

has a span of 0.6 meters, clearance 0.3 meters; Bridge #4 has a span of 0.3 meters, clearance 0.15 meters.

The bridges are in a lens of fine-grained inosculated anastomosing silicestone (very similar to Flaser bedding in sandstones). The silicestone is capped by beds of cherty limestone of wackestone almost a packstone (Dunham's classification). Two major joint trends occur at the bridge site; N41°E and N55°W. The bridge complex trends N36°E and is structurally controlled by the major joint sets.

Assessment of Sinkhole Collapse Hazards for Insurance Purposes: A Model for Tennessee. F. B. BOOTHE and P. R. KEMMERLY, Austin Peay State University.

Four of the five largest cities in Tennessee are underlain by soluble carbonates, where sinkhole collapse is not uncommon. Sinkhole collapse is primarily triggered by disruption of surface drainage patterns and artificial fill placed in sinkholes during urbanization.

Using Clarksville, Tennessee as a case study, sinkhole-collapse risk maps were prepared for an area in east Clarksville currently experiencing rapid urbanization. Collapse risk criteria included sinkhole length/width ratio and long-axis orientation with respect to joints. Previous investigations indicated that long-axis orientation and length/width ratio and long-axis ratio are strongly correlated with sinkhole collapse. Both indices reflect the relative solution-enlargement of systematic joints and efficient ground-water movement.

Sinkholes were designated as high, moderate, or low risk based on length/width ratio relative to 1.80 and sinkhole long-axis orientation with respect to systematic joint sets. The classification for the three risk categories are: high risk: length/width ratio less than 1.80 and sinkhole long-axis parallel to the N. 70°-80° E. joint set. Moderate risk, length/width ratio greater than 1.80 and long-axis orientation parallel to the N. 70°-80° W. joint set or length/width ratio less than 1.80 and the long-axis orientation parallel with the N. 70°-80° E., N. 20°-40° E., or N. 20°-30° W. joint sets. Low risk, length/width ratio greater than 1.80 and long-axis orientation not parallel to any systematic joint sets.

As a result of this study, Tennessee home insurers are currently investigating the feasibility of sinkhole collapse protection.

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EXPERIMENTAL INVESTIGATIONS INTO THE NON-ENZYMATIC ORIGIN OF THE CITRIC ACID CYCLE

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ABSTRACT

Prompted by published speculations that the citric acid cycle originated in simple organisms prior to the buildup of biomonomers on the primitive Earth, experiments were performed to discover if Krebs cycle reactions have a tendency to occur without catalysts under primordial Earth conditions. With one exception (the thermal isomerization of citric to isocitric acid), the results of these experiments did not indicate such a tendency. These findings, when considered along with classical chemical evolution experiments, support the view that the Krebs cycle evolved late, in an already complex environment.

INTRODUCTION

Although the citric acid (Krebs) cycle has long been recognized for its central role in aerobic metabolism (Stryer, 1975), the question of its origin and evolution has not been the subject of a systematic experimental approach. Some bases for speculation are provided by chemical evolution experiments which produce acetic acid and succinic acid (Krebs cycle members) along with numerous other biological molecules such as amino acids, fatty acids, and carbohydrates (Calvin, 1969). In addition, it has been shown that synthetic protenoids catalyze the decarboxylation of α -ketoglutaric acid, an important step in the modern Krebs

cycle (Fox et al., 1970). Other experiments (Lemmon, 1970) tend to support the view that metabolic reactions originated within a simple organism in an already complex "primordial soup" environment.

In contrast, Hartman (1975) has proposed a scenario in which "the citric acid cycle came first and was followed by the amino acids, lipids, nucleotides, and carbohydrates." Indeed, the oxidation, elimination, and addition reactions currently observed to take place in the cycle could all, in principle, take place under oxidative conditions on the primitive Earth. With Hartman's unique speculation in mind, the present study was initiated to discover if the reactions of the citric acid cycle have a propensity to occur thermally or under the influence of ultraviolet light and without complex catalysts such as enzymes or synthetic polypeptides. The reaction conditions used in this work are in accordance with evidence summarized by Fox and Dose (1977) that ultraviolet light and temperatures up to 300 degrees C would have been common sources of energy on the surface of the primordial Earth. If the citric acid cycle evolved early in the origin of metabolism (Hartman, 1975), one might expect the cycle reactions to occur under primitive Earth conditions.

METHODS

All thin-layer chromatograms were prepared using Eastman Chromagram sheets (silica gel, 0.2 mm thickness). Plates were eluted with a water-saturated solution of ether and 85% formic acid (7:1) and were visualized in an iodine saturated chamber. Aqueous solutions were prepared with line distilled water redistilled from KMnO_4 solution.

Thin-Layer Chromatographic Separation of Citric Acid Cycle Intermediates (Beaudoin et al., 1973)

Dilute aqueous solutions of citric, isocitric, alphaketoglutaric, succinic, fumaric, and malic acids were separated on TLC plates. The results are summarized in the Figure. Decomposition of oxalacetic acid (OAA) during chromatography was continually observed, precluding its identification in subsequent experiments. The various products which were observed in the below experiments do not correspond on TLC to any of these OAA decomposition products nor do the OAA decomposition products correspond to Krebs cycle compounds. Contrary to the report by Beaudoin et al. (1973) that OAA can be chromatographed without decomposition on silica gel plates impregnated with MgSO_4 and Na_2CO_3 , it was not possible to avoid this problem using the published method.

Attempted Aldol Reaction Between Acetic and Oxalacetic Acids

A solution containing 5 mg of OAA, 3 drops of glacial acetic acid, and 5 drops of water was left in a test tube overnight at room temperature. The solution was boiled to dryness and a chemical spot test (Feigl, 1966) for detecting citric acid (2 microgram minimum) was performed. This test, involving UV fluorescence of ammonium citrazinate formed by fusing citric acid with urea, indicated that citric acid was not present in the solid reaction product.

Decomposition of Citric Acid Under Primitive Earth Conditions

Aqueous solutions, 100 degrees C—The following solutions (volume = 1 mL) were prepared in distilled water and heated for three hours at 100 degrees C in open tubes: (1) 1 M citric acid, (2) 1 M citric acid containing 6 drops of 0.5 M MgCl_2 , (3) 1 M citric acid containing 6 drops of 0.5 M FeCl_3 , (4) 1 M citric acid adjusted to pH 6.7 with Na_2CO_3 , (5) solution 2 adjusted to pH 6.7 with Na_2CO_3 , (6) solution 3 adjusted to pH 6.7 with Na_2CO_3 . After three days at room temperature, TLC analysis showed only citric acid to be present in each solution with no decomposition products visible.

Aqueous solution, ultraviolet light—On a spot plate, 1 mL of a 1 M aqueous solution of citric acid was exposed to a UV light source (366 nm, 400 W/cm² at 15 cm) at a distance of approximately 0.5 cm for 12 hours. Resulting decomposition products were shown not to be Krebs cycle intermediates (see Figure).

Neat, pyrolysis—Granular citric acid (12.6 g) was heated over a Bunsen burner to 140 degrees C for 45 minutes. The remaining dark, amber fluid (5.3 g) showed strong infrared absorption at 1760 cm^{-1} and 1850 cm^{-1} (carboxylic acid anhydrides). A portion of this product was hydrolyzed in boiling dilute HCl for 1 hour and analyzed by TLC (Figure). Isocitric acid was identified among the products.

Pyrolysis of Alphaketoglutaric Acid—

Alphaketoglutaric acid (2.9 g) was heated to 200-215 degrees C for 30 minutes. The resulting dark, viscous liquid (2.5 g) was hydrolyzed by boiling with dilute HCl. The products, separated by TLC (Figure 1), were not Krebs cycle compounds.

Pyrolysis of Succinic Acid—

In a parallel experiment, succinic acid was boiled for 30 minutes at temperatures from 205-260 degrees C. The clear viscous fluid crystallized on cooling and was shown by TLC to be pure, recovered succinic acid.

RESULTS AND DISCUSSION

The first citric acid cycle reaction which was tested for its ability to take place under primordial conditions was the initial Aldol reaction between acetic and oxalacetic acids. An aqueous solution of these reactants was left overnight at room temperature. After evaporation of the solution (100 degrees C) the dry residue was analyzed for citric acid according to the method reported by Feigl (1966). No citric acid product was detected using this test which is sensitive to 2 micrograms of material.

The behavior of citric acid under primitive Earth conditions was studied. A 12 hour exposure to ultraviolet radiation decomposed an aqueous solution of citric acid to form two products, neither of which is a Krebs cycle intermediate (see Figure). Separate dilute aqueous solutions of citric acid were prepared: (1) pH 1.5, (2) pH 6.7, (3) with Mg^{+2} , (4) with Fe^{+3} . After heating each solution at 100 degrees C for several hours in the presence of air, no decomposition of the citric acid was detected on a thin-layer chromatogram. Finally, crystalline citric acid was pyrolyzed at 140 degrees C for 45 minutes in an open test tube. After hydrolysis of anhydrides, products were seen on a thin-layer chromatogram (Figure). With the exception of isocitric acid, none of the pyrolysis products were Krebs cycle intermediates. The remarkable thermal isomerization of citric to isocitric acid has been reported by Usoltseva et al. (1970) and presumably occurred by elimination of water from citric acid followed by addition in the opposite sense across the intermediate double bond. This was the only non-enzymatic Krebs cycle reaction which was observed under primitive Earth conditions. The meaning of this event in terms of chemical evolution is not clear at this time.

A similar pyrolysis of alphaketoglutaric acid also produced products which were observed on TLC (Figure) after hydrolysis to convert anhydrides to carboxylic acids. No Krebs cycle intermediates were produced. Succinic acid, upon pyrolysis and hydrolysis, yielded only pure starting material according to TLC analysis. Air oxidation to fumaric acid and subsequent reactions did not occur.

The plethora of biological molecules, including the Krebs cycle members acetic and succinic acids, produced under primitive Earth conditions (Calvin, 1969), created a potential for prebiotic chemical reactivity. Were complex catalysts needed for the rudiments of