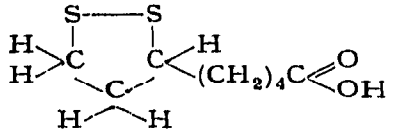
CHROM. 4244

The use of thin-layer chromatography in following product formation in the photolysis of α-lipoic acid*

In a study of the effect of solvent on the photolysis of α-lipoic acid (1,2-dithiolane-3-valeric acid)[1,2]



it was found that analytical thin-layer chromatography (TLC) could be used to follow the formation of products. The analytical procedures are described as well as the preparative TLC methods that were employed to isolate several of the products of the photolysis reaction in diethyl ether. An outgrowth of this work was the quick method for determining the presence of sulfhydryl groups, which was reported earlier[3].

Materials

All solvents used in the photolysis were either spectral grade (where possible) or reagent grade. The diethyl ether, methanol and isopropanol used were either freshly distilled or were taken from containers that had been opened just prior to use so that peroxides would not be present. The α-lipoic acid (LA) and dihydrolipoic acid (DHL) were obtained from Sigma Chemical Company. The commercial samples of LA were recrystallized several times but this purification did not change the LA in any way. Therefore, succeeding samples were not recrystallized. All adsorbents were purchased from Brinkmann Instruments Company.

Analytical TLC

Plates (20 × 20 cm) were coated with a 0.50 mm thick layer of Silica Gel HR. Eight samples of 20 μl each were analyzed on a plate at one time. A solvent system of chloroform–methanol–formic acid (8:1:1) was used to develop the plates. Iodine was the visualizing agent. Typical analytical plates are shown in Fig. 1.

Preparative TLC

The plates (20 × 20 cm) were coated with a 2 mm thick layer of Silica Gel PF_{254}. For applying the samples to the plate, a technique suggested by BOBBITT[4] was used. This technique consisted of using a doubly-bent 1 ml pipette inserted into a small paint brush. This allows even application of a band of 1 ml of a 2×10^{-2} *M* solution. After the solvent had evaporated from the band, another milliliter of sample was applied over the first. This procedure was repeated five times. The plates were developed three times in the solvent system described above. For the preparative work, it was preferable to remove the photolysis products from the plates without

* This material was submitted as part of P.R.B.'s Ph. D. Thesis to Brown University, June, 1968.

exposing them to the iodine. Therefore, a "hot wire" (heated electrically to a red glow) was applied at the side of the plate[1]. Charring occurred on the plate at the position of the bands. After the band had been removed (using a Brinkmann "micro vacuum spot remover"), the plate was visualized to make sure that the product removed was not contaminated with a part of another band. A typical preparative TLC plate in which the bands were located by the hot wire technique is shown in Fig. 2 and one of the preliminary plates in which the bands were visualized with iodine is shown in Fig. 3.

The lipoic acid photolysis products were extracted from the adsorbent by

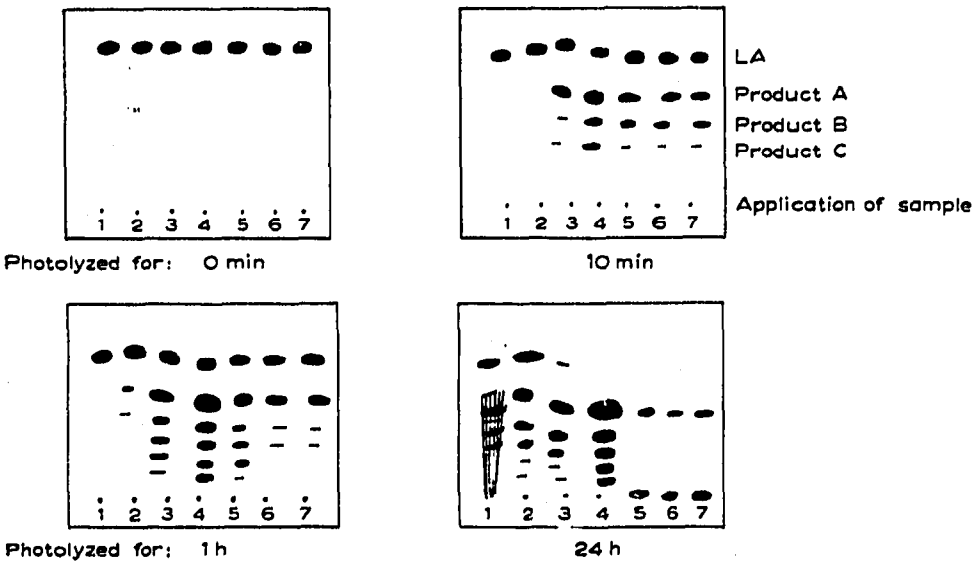


Fig. 1. Analytical TLC plates of photolyzed solutions of LA in various solvents. Solvent: 1 = 95% ethanol; 2 = isopropanol; 3 = chloroform; 4 = diethyl ether; 5 = benzene; 6 = cyclohexane; 7 = carbon tetrachloride.

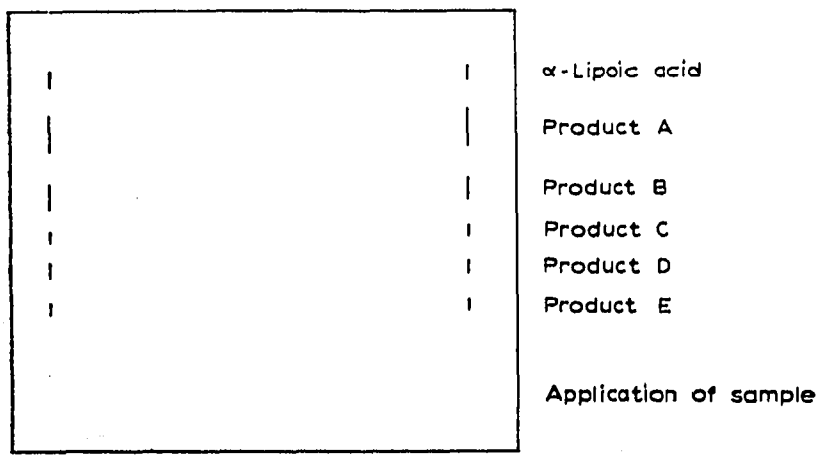


Fig. 2. Preparative TLC plate of a photolyzed diethyl ether solution of LA showing visualization with the "hot wire" technique.

washing the gel with diethyl ether, filtering off the solid and testing the solution for product purity on analytical TLC.

By testing LA and DHL on analytical TLC, it was found that both compounds had the same R_F value but it was noted that the LA spot turned dark brown immediately whereas the DHL spot, which was more diffuse, first bleached the tan color of the plate and then became dark brown. It was also noted on visualizing the TLC plates with iodine that many of the products of photolysis bleached the iodine and formed a white spot at first. Only after exposure to the iodine for about 5 min did these spots turn the same dark brown as the LA spot. On trying reference compounds containing carboxyl and sulfur functional groups, it was found that the bleaching action was due to the presence of sulfhydryl groups. Apparently these groups reduced the iodine to iodide ion.

$$2\ RSH + I_2 \rightarrow R\text{–}S\text{–}S\text{–}R + 2H^+ + 2\ I^-$$

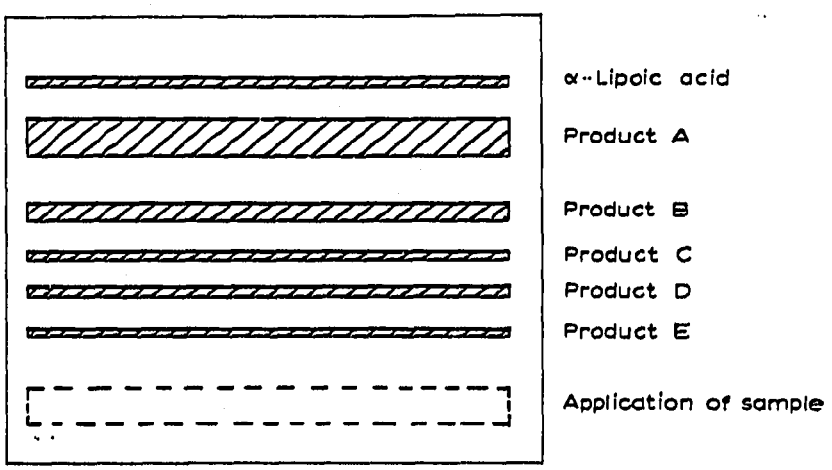


Fig. 3. Preparative TLC plate of a photolyzed diethyl ether solution of LA showing visualization with iodine.

The disulfide compounds thus formed complexed with the iodine only after all the sulfhydryl was oxidized. This phenomenon was used as a quick, qualitative test for sulfhydryl groups in the presence of other sulfur compounds[3].

Photolysis

For the first series of reactions, the solvents in which the LA was photolyzed were as follows: 95% ethanol, isopropanol, chloroform, diethyl ether, benzene, cyclohexane, carbon tetrachloride, hexafluorobenzene, and water, OH^-. The photolyses in hexafluorobenzene and diethyl ether were run both under nitrogen and in air to determine the effect of oxygen on product formation. In the second series, the effect of water on the photolysis of LA in ethanol was observed. For the third series, the LA was dissolved in the various alcohols (water-free). All the photolysis reactions were followed by TLC.

Fresh solutions of LA were prepared for each photolysis run. The UV source used was an 8 W Sylvania Blacklight Blue UV tube lamp (No. F8T5/BLB) which radiates the major portion of its energy in the ultraviolet, peaking the 3560 Å region. It was assumed for our semi-quantitative studies that the glass vials were identical in spectral

characteristics and that "end-effects" relative to lamp position were not significant.

To follow the development of products with time, 10 ml of a solution of LA in each solvent were prepared. Five-milliliter samples of each solution were placed in Pyrex weighing bottles with ground glass stoppers and photolyzed as above. At the specified time intervals, 20 μl of each sample were removed and spotted on an analytical plate. Therefore, each plate contained samples of each solution photolyzed for the same length of time. The time intervals were 0, 10, 20, 30, 45, 60 and 120 min and 24 h for the first series and 0, 15, 30, 45, 60, 120 and 240 min and 24 h for the second and third series.

Results

The analytical plates in Fig. 1 show the formation of products with time. The products were designated A, B, C etc. until they could be identified. Product A was the product that had the highest R_F value (except in the alcohols wherein a small amount of an unidentified compound designated A' was observed), B next lower, etc. No product had an R_F value higher than that of LA. The product with an R_F value of zero was designated as polymer as it was found that increasing molecular weights caused decreasing R_F values. It should be noted that the fact that two compounds have the same R_F value does not necessarily mean that they have identical compositions. However, because of the distinct nature of the spots on TLC, they must be similar. Therefore, the product we call Product A in diethyl ether may differ in detail from Product A in hexafluorobenzene.

It was found that good separation of TLC spots was obtained by the use of analytical TLC in following the photolysis of LA in organic solvents but poor separation was obtained for the samples from aqueous solutions. Preparative TLC proved satisfactory for the separation of photolysis products in the diethyl ether solution. Product A was formed in this solution in approximately 50 % yield and its structure was elucidated on the basis of UV, IR, DTNB sulfhydryl determinations[5], elemental analysis, molecular weight determination and quantitative determination of the products formed in the reaction of base with photolysis products[1]. This evidence indicated that Product A was a linear sulfhydryl dimer and is probably a mixture of closely similar structural and stereoisomers. The other products formed in this solution were oligomers of varying molecular weights.

Metcalf Chemical Laboratories, Brown University, Providence, R.I. 02912 (U.S.A.)

PHYLLIS R. BROWN*
JOHN O. EDWARDS

1 P. R. BROWN, *Ph. D. Thesis*, Brown University, Providence, R.I., 1968.
2 P. R. BROWN AND J. O. EDWARDS, in press.
3 P. R. BROWN AND J. O. EDWARDS, *J. Chromatog.*, 38 (1968) 543.
4 J. BOBBITT (University of Connecticut), personal communication, 1966.
5 G. L. ELLMAN, *Arch. Biochem. Biophys.*, 82 (1959) 70.

Received June 18th, 1969

---

* Union Carbide Fellow, 1966–67. N.I.H. Special Research Fellow, 1967–68 (Grant No. 1-Fl-GM-35-937-01).

Present address: Department of Biomedical Sciences Brown University, Providence, R.I., U.S.A.