enzyme coenzyme complex[5–7]. If this is so the observations reported here allow the supposition that the coenzyme is bound more firmly to LDH_1 than to LDH_5, so protecting the tertiary structure of the enzyme molecule necessary to catalytic action.

The elevated pyruvate concentration increases the reaction velocity of urea-treated LDH_1. This consequently means that the affinity of the enzyme towards its substrate is lowered possibly as a result of changes in the conformation of enzyme molecule in its active centre caused by urea.

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Preparation of Endotoxin

When a watery suspension of Gram-negative organisms is heated at 80° C for 1 h, the endotoxin is liberated into the suspending fluid, giving it a dichroic appearance; a method of preparing endotoxin on a large scale by recovering it from such a supernatant was described earlier[1]. In that method concentration was effected by evaporation under reduced pressure. This method, improved and modified to avoid this, is described here.

A culture is grown in a medium containing commercial-grade ammonium sulphate 0·5 g, glucose 1·0 g, disodium hydrogen phosphate 0·2 g, sodium citrate 0·2 g, and magnesium sulphate 0·02 g in 100 ml. of distilled water. A still culture in this medium with the *p*H held at 7·5 yields 3×10^9 and an aerated culture 10^{10} viable cells per ml. The cultures are stored at 4° C, and when there is a convenient quantity available they are centrifuged to obtain the cells in a stiff paste; the cells are re-suspended in distilled water to about 10^{11} cells/ml. and the suspension is heated at 80° C for 1 h and centrifuged. The cells are discarded and the oil-like supernatant is dialysed against running tap-water for 72 h to remove amino-acids, and the endotoxin precipitated in 75 per cent alcohol and dried. It is a slightly greyish sugar-like powder.

Greater purity can be achieved by (*a*) re-suspending the living cells in chilled water and repeating the centrifuging, (*b*) continuing the dialysis against distilled water, and (*c*) re-precipitating in 75 per cent alcohol.

In a typical batch the yield of endotoxin from 10^{15} cells of *E. coli* was 7 g and the LD_{50} of this by intraperitoneal injection in 18–22 g mice was 0·5–1·0 mg.

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Depsides in Sunflower Leaves

This communication reports our finding of neochlorogenic, isochlorogenic, and 3-*O*-feruloylquinic acids in the leaves of sunflowers, *Helianthus annuus*, Russian Mammoth variety. The well-known chlorogenic acid (3-*O*-caffeoylquinic acid) has been previously found in sunflowers by Urban[1] and its presence there has been confirmed by Watanabe *et al.*[2].

Neochlorogenic acid, first isolated from peaches by Corse[3], has recently been proved to be 5-*O*-caffeoyl-D-quinic acid[4–6]. Isochlorogenic acid, as available commercially, has been shown to be a mixture of depsides[4–7]. Corse *et al.*[6] have identified one component as 4,5-di-caffeoyl-D-quinic acid, and Haslam *et al.*[5] have identified another component of the mixture as 3,5-dicaffeoyl-D-quinic acid. The isochlorogenic acid from sunflowers contains three components identical with three of the components, other than chlorogenic acid, of commercially available reference isochlorogenic acid. Neochlorogenic acid and isochlorogenic acid have previously been found in coffee beans, blueberry leaves, plums, and other fruits[8], but not in sunflowers. Recently, 3-*O*-feruloyl-D-quinic acid has been isolated as a crystalline material from unroasted coffee beans[9], but had not been found previously in sunflower plants.

Isolation and identification of neochlorogenic acid. Sunflower leaves (194 g) grown in the greenhouse of the Department of Botany, University of Oklahoma, were extracted by twice boiling the disintegrated leaves with 1 l. portions of 85 per cent isopropyl alcohol–water for 5 min. The extracts were filtered, concentrated *in vacuo* at 37° C to 250 ml. and extracted three times with benzene. The aqueous concentrate was poured on to a 4·5 × 10 cm chromatographic column which previously had been packed with 'Magnesol' (Food Machinery and Chemical Corp, N.Y.) and washed with distilled water under a pressure of 5 lb./in.^{2} pressure. The yellow zone on the column was developed with distilled water to produce two major zones, called fraction *A* and fraction *B*, which were eluted off separately. Fraction *A*, the faster moving zone, was streaked on to ten sheets of Whatman 3 *MM* paper and developed in *n*-butyl alcohol–acetic acid–water (6 : 1 : 2 v/v/v, here referred to as BAW). The broad zone at R_F 0·75 was cut out and eluted with methanol–water (1 : 1 v/v). The extracts were concentrated and streaked on ten sheets of paper and developed in butyl acetate–acetic acid–water (4 : 1 : 5 v/v/v) for 20 h. The broad zone at R_F 0·70 (chlorogenic acid) was separated from a narrow one at R_F 0·25. The latter zone was eluted and further purified by streaking the eluate on seven sheets of Whatman 3 *MM* paper and developing the chromatograms in BAW. This procedure was repeated on nine sheets of Whatman 3 *MM* paper, and then on eight sheets of Whatman No. 1 paper in isopropyl alcohol–formic acid–water (50 : 1 : 950 v/v/v, here referred to as IFW). The extract was taken to dryness *in vacuo*, dissolved in a small amount of methanol and poured on top of a 2·5 × 25 cm 'Magnesol' column previously packed under a pressure of 5 lb./in.^{2} with distilled methanol. The column was developed with one column length of methanol followed by methanol–water (1 : 1 v/v). The zone which fluoresced greenish blue under ultra-violet light (3660 Å) was collected. The absorption spectrum of the eluate showed a maximum at 326 mμ, shoulders at 242 and 300 mμ, and a minimum at 265 mμ. The eluate was taken to dryness and hydrolysed with 10 ml. of 5 per cent sodium hydroxide for 20 min at room temperature. The solution was then acidified with concentrated hydrochloric acid and extracted three times with ethyl acetate. The ethyl acetate extract was chromatographed on Whatman No. 1 paper to prove the presence of caffeic acid. After the evaporation of the aqueous phase, the dry crystalline residue was extracted with 5 ml. absolute ethyl alcohol. The alcohol extract on chromatography showed the presence of quinic acid. The hydrolysis products, the R_F values (Table 1), and the ultra-violet spectra on comparison with authentic neochlorogenic acid showed that the compound extracted was identical with the reference compound.

Isolation of isochlorogenic acid. Fraction *B*, the slower moving zone from the 'Magnesol' column, was concentrated *in vacuo* and streaked on 20 sheets of Whatman 3 *MM* paper and developed in IFW to give three zones. The broadest and most intense one under ultra-violet light at R_F 0·22 was cut out and eluted with 70 per cent isopropyl alcohol. The eluate was concentrated to a