and sunflower seed oil. The content of triene was determined after calculation of k'_3 by eqn. I or eqn. II and F_3^{sample} was calculated by eqn. IV. It is seen that F_3^{sample} is consistently higher than 4.12 indicating that a background absorption has developed during isomerization. In accordance with this, calculation of k'_3 by eqn. I gives higher values than by eqn. II.

In order to determine whether the use of eqn. II, when F_3^{sample} is higher than 4.12, will give the *correct* value for % triene, a number of recovery experiments were carried out. Known mixtures of soybean oil and linoleic or linolenic acid were isomerized and % triene determined after calculation of k'_3 by eqn. I or II. F_3^{sample} was calculated by eqn. IV. The results in Table 3 confirm, that when F_3^{sample} is higher than 4.12, as seen in the isomerized mixtures of soybean oil and linoleic acid, calculation of k'_3 by eqn. II gives the correct value for % triene.

In conclusion it can be said that the value of F_3^{sample} will indicate which of the eqns. I or II will give the correct value for k'_3 and thereby the correct value for the triene content of the sample. If F_3^{sample} is higher than 4.12 eqn. II should be used, in all other cases eqn. I will give the correct k'_3.

In an attempt to standardize the spectrophotometric determination of polyenoic fatty acids and to simplify the calculations involved, Brice *et al.*[4] have recommended the use of eqn. II only when k'_{268} is lower than 1. In Table 4 the values for k'_{233} k'_{268}, k'_{233}/k'_{268} and F_3^{sample} found in the experiments described in this paper are collected. It is clearly seen that in several experiments, in which k'_{268} is greater than 1, eqn. II must be used since F_3^{sample} is higher than 4.12. From Table 4 it furthermore appears that if the ratio k'_{233}/k'_{268} is higher than 7, F_3^{sample} is higher than 4.12. Inspection of spectral curves for isomerized samples of this type clearly shows that the diene peak, with maximum at 233 mμ, contributes significantly to the absorption in the region 262—274 mμ. As this "tail absorption" is approximately linear, eqn. II will give the true k'_3 for these samples.

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Studies on Rennin

IV. Chromatographic Fractionation of Rennin

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In previous papers of this series rennin was considered a rather homogeneous protein. Only one peak was observed on moving boundary electrophoresis at pH 5.8, and in a solubility test the enzyme behaved nearly as a single-component system (Foltmann [1,2]).

On the other hand, Ernstrom [3] reported that although rennin only showed one peak on electrophoresis in buffers of ionic strength 0.1—0.2, it would split up in 3—4 peaks on moving boundary electrophoresis in buffers of ionic strength 0.033 and pH 6.8. In a personal communication to the author, Ernstrom added that on zone-electrophoresis in buffers of ionic strength 0.033 he had observed a slight displacement of enzymatic activity as compared with optical density; however, no separation into discrete peaks was achieved by such zone-electrophoresis.

Jirgensons *et al.*[4] have reported chromatography of a commercial crystalline rennin on diethylaminoethylcellulose. They found that the rennin could be fractionated into one inactive and two active fractions. However, these experiments were carried out at pH 6.7, which is somewhat above the stability range of the enzyme (Foltmann [1]).

The present communication deals with fractionation at a more appropriate pH of rennin by chromatography on diethylaminoethylcellulose (DEAE-cellulose) as described by Peterson and Sober [5]. The ion-exchange cellulose used was a commercial

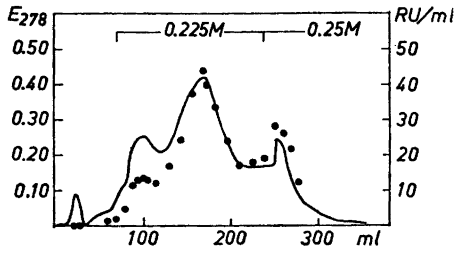

Fig. 1. Chromatography of dissolved rennin crystals on DEAE-cellulose, using stepwise elution with phosphate buffers pH 5.7; 0.20 M, 0.225 M and 0.25 M. Load 40 mg. Column 0.9 × 17 cm. Flow rate 13.5 ml/h, collected in fractions of 4.5 ml. ● milk-clotting activity (RU/ml). Experimental points have been omitted from the extinction curve (E_{278})for the sake of clarity.

product (Eastman-Kodak, lot no. 7392). The rennin was prepared and recrystallized as earlier described [1]. After the crystals had been dissolved by dialysis of the suspending medium (4 M NaCl), the sample was applied to a column of DEAE-cellulose previously equilibrated with 0.20 M phosphate buffer of pH 5.7. The elution was carried out by stepwise increase of the phosphate concentration to 0.225 M and 0.25 M. Fig. 1 illustrates one of the experiments. In the figures the concentration of protein is expressed by the optical density at 278 mμ. The enzymatic activity is expressed in rennin units (RU) according to Berridge [6].

The chromatogram shows a slight front peak of inactive material. After change of the buffer concentration to 0.225 and further to 0.25 M phosphate, a complex system of three peaks develops. Each of the three peaks contains milk-clotting activity, but the specific activity, *i.e.* the ratio RU/E_{278} is different for the three peaks. The component which is eluted last has the highest specific activity; RU/E_{278} equals about 125. This fraction is called the A-rennin. The main component which is called B-rennin has a specific activity of about 100, while the first eluted component, the C-rennin, has a specific activity of 55—60. The recovery of milk-clotting activity was 98 % and on an optical density basis the recovery was 102 %. Calculated from optical densities the first peak of inactive material represents *ca.* 3 % of the total, while the

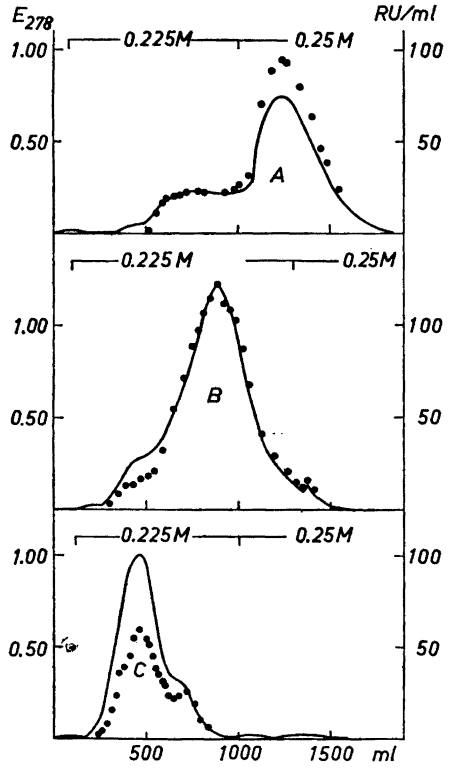

Fig. 2. Rechromatography of pooled fractions containing A, B, and C rennins, respectively. Columns of DEAE-cellulose 2 × 23 cm. Stepwise elution with phosphate buffers pH 5.7; 0.20 M, 0.225 M and 0.25 M. Load 160 to 300 mg per run. Flow rate 25—57 ml/h, three fractions collected per hour. ● milk-clotting activity (RU/ml). Solid line, optical density at 278 mμ.

A, B, and C peaks represent 22, 55, and 20 %, respectively.

For each of the three components, the corresponding fractions from several runs were pooled, and after concentration each component was further analysed and purified by rechromatography. Fig. 2 illustrates three such experiments involving rechromatography of pooled fractions. In these chromatograms the main peaks appear at three different positions corresponding to the A-, B-, and C-rennins, thus indicating that peak formation in the first

run with the crystalline material is not due to artefacts arising during chromatography. Furthermore the specific activities of the purified components also agree with those found in the peaks obtained in the first run of rennin crystals. In each of the A- and B-peaks, the specific activity is constant (within experimental error) across the peak, but over the C-peak there is a slight increase in specific activity. Whether this is due to overlapping contamination with B-rennin, or whether the C-rennin is a mixture itself is not yet clear.

The experiments indicate that the crystalline rennin investigated contains only small amounts of inactive material, and further that the crystals contain at least three components with different enzymatic activity.

Examination of the results of Jirgensons *et al.*[4] shows that the main component in their chromatogram has increasing specific activity over the peak, suggesting that this peak in fact represents a mixture of A- and B-rennins. This assumption is supported by the failure of attempts to separate A- and B-rennins by chromatography at pH 6.2 in this laboratory.

Full details of these experiments and further studies on the rennin fractions will appear in the *Comp. rend. trav. lab. Carlsberg.*

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Enzymatic Hydrolysis of Bifunctional S-Substituted L-Cysteine Derivatives

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Previous publications [1,2] have reported that the C—S-lyase of the endosperm of the seeds of *Albizzia lophanta* can act on a variety of S-substituted cysteine and cysteine sulfoxide derivatives. Whereas a typical monofunctional derivative such as S-ethyl-L-cysteine can undergo complete hydrolysis to pyruvic acid [3], results presented in this communication show that the two bifunctional cysteine derivatives investigated, djenkolic acid and L-lanthionine sulfoxide, are not fully hydrolyzed. The extent of hydrolysis of djenkolic acid, as measured by formation of pyruvic acid, amounted to 66.0, 67.2, and 65 % at substrate concentrations of 1, 2, and 2.5 mM, respectively (Fig. 1). Thus the actual maximum amount of pyruvate formed was pro-

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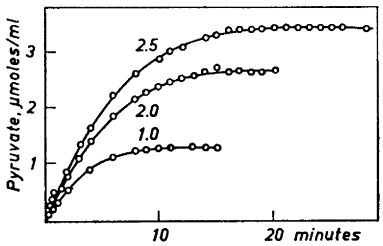

Fig. 1. Course of hydrolysis of djenkolic acid by *Albizzia* C—S-lyase. One ml of reaction mixture at 37° contained 35 μg of enzyme (specific acitivity = 200)[4], 40 μmoles of borate buffer, pH 8.0 and 0.02 μmole of pyridoxal phosphate. Micromoles per ml of djenkolic acid are indicated for each curve.