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Hydrogenation of Reduced Aldol Condensation Product from Elastin*

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ABSTRACT: The catalytic hydrogenation of the reduced aldol condensation product isolated from reduced bovine elastin yields several products. Isolation of these products was achieved by ion-exchange chromatography. The structures of the most abundant products were determined by mass spectrometry. Results obtained were consistent with the presence of an allylic alcohol group in the previously isolated reduced aldol condensation product.

Lent *et al.* (1969) have reported the presence of a reduced aldol condensation product (ACP;[1] I) in alkaline hydrolysates of elastin previously reduced with $NaBH_4$. Available data suggest that this compound arises in elastin from the condensation of two residues of α-aminoadipic acid δ-semialdehyde, the latter resulting from the enzymatic deamination of lysyl residues previously incorporated into the polypeptide chains of elastin (Salcedo *et al.*, 1969).

$$\underset{\text{I}}{HOOCCH(NH_2)(CH_2)_2C(CH_2OH)=CH(CH_2)_3CH(NH_2)COOH}$$

Previous evidence presented for the structure of the reduced ACP included the mass spectrum of its ethyl ester derivative as well as studies on the periodate–permanganate oxidation of the compound (Lent *et al.*, 1969). However, the position of the double bond has not been clearly established.

This communication presents additional studies on the catalytic hydrogenation of the reduced ACP. The data support the presence of an allylic alcohol in the structure proposed.

Experimental Section

Isolation of the reduced ACP from elastin was described previously (Lent *et al.*, 1969). The reduced ACP used in these experiments was isolated from 200 mg of bovine elastin, which had been previously reduced with $NaBH_4$. The elution pattern of the reduced ACP from the Technicon amino acid analyzer empolying the gradient of Burns *et al.* (1965) is given in Figure 1.

Hydrogenation was performed in a Parr apparatus as follows. Approximately 1.0 μmole of reduced ACP from elastin was dissolved in 0.6 ml of 0.1 N HCl. To this was added 5.0 ml of H_2O and 15 mg of palladium black (Fisher Scientific Co., Lot 752241). Hydrogenation was then carried out in 2 atm of H_2 gas for 18 hr at room temperature. The reaction mixture was filtered and evaporated to dryness. The residue was dissolved in 1.0 ml of 0.01 N HCl.

Separation of the reaction products from the hydrogenation experiment was achieved by use of the Technicon amino acid analyzer employing the gradient described by Burns *et al.* (1965). A typical chromatogram of the reaction mixture is also shown in Figure 1. All ninhydrin-positive peaks were arbitrarily given a designated letter, A through E. In the several experiments performed, compound A and compound E were the major ninhydrin-positive fractions which appeared on the amino acid analyzer after hydrogenation. Together they accounted for 75–80% of the total ninhydrin reactivity recovered from the reaction mixture; compound A varied from 10 to 25% of the total ninhydrin, while compound E varied from 50 to 75%. Isolation of the individual compounds was accomplished by employing a stream-splitting device. Pooled fractions were desalted on a Dowex-50 column employing 0.25 M NH_4OH as the elution buffer.

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[1] Abbreviation used is: ACP, dehydrated aldol condensation product of two residues of α-aminoadipic acid δ-semialdehyde.

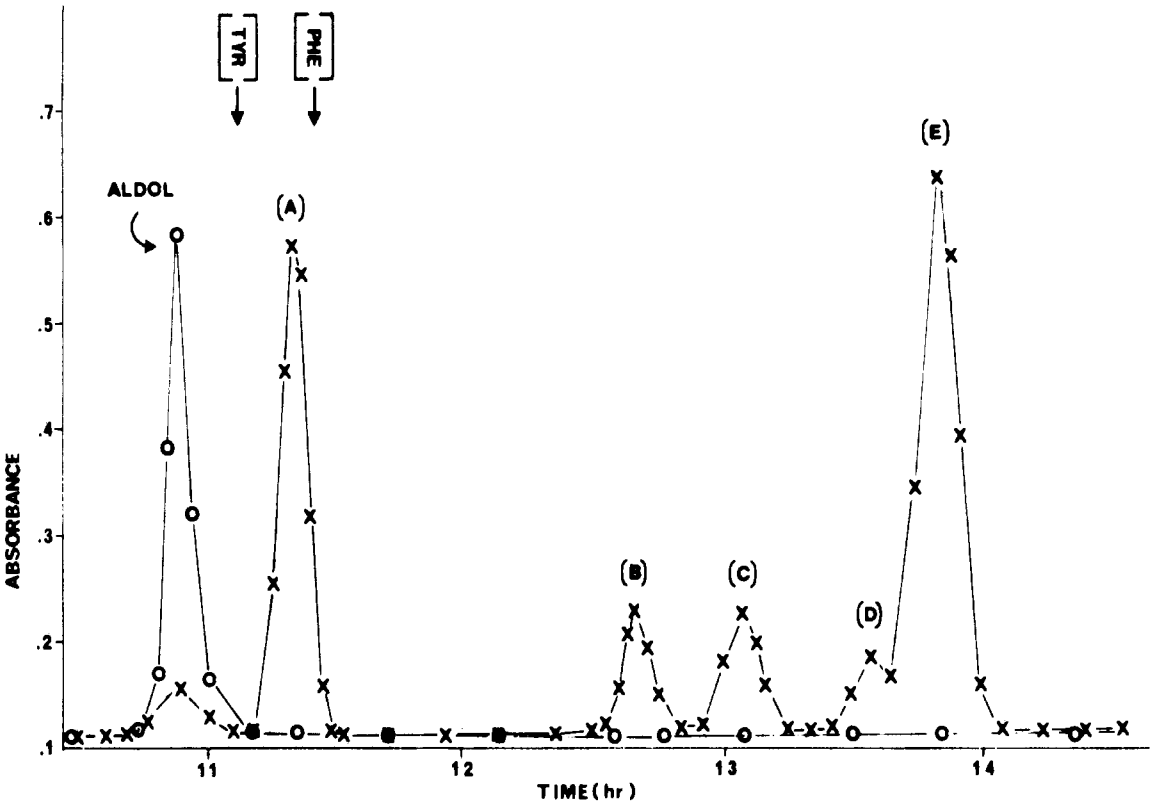

FIGURE 1: Ion-exchange chromatography of the reduced ACP before (O——O) and after (×——×) catalytic hydrogenation. Separation was performed on a Technicon amino acid analyzer equipped with a stream-splitting device. Ninhydrin reactivity was monitored at 570 mμ. The gradient employed was that of Burns *et al.* (1965). Arrows indicate positions of certain amino acids on amino acid analyzer.

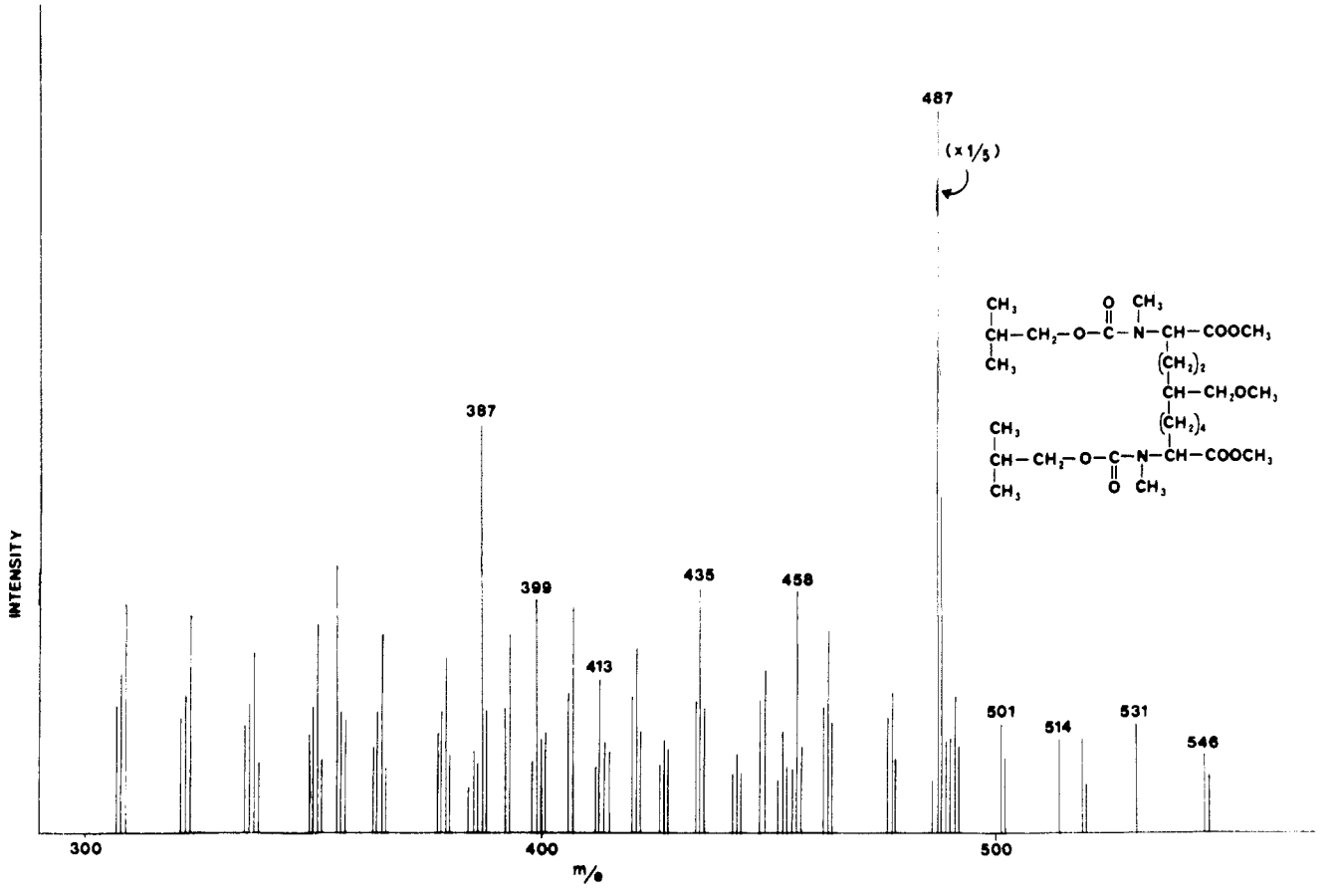

FIGURE 2: Mass spectrum of Iboc-permethylated compound A.

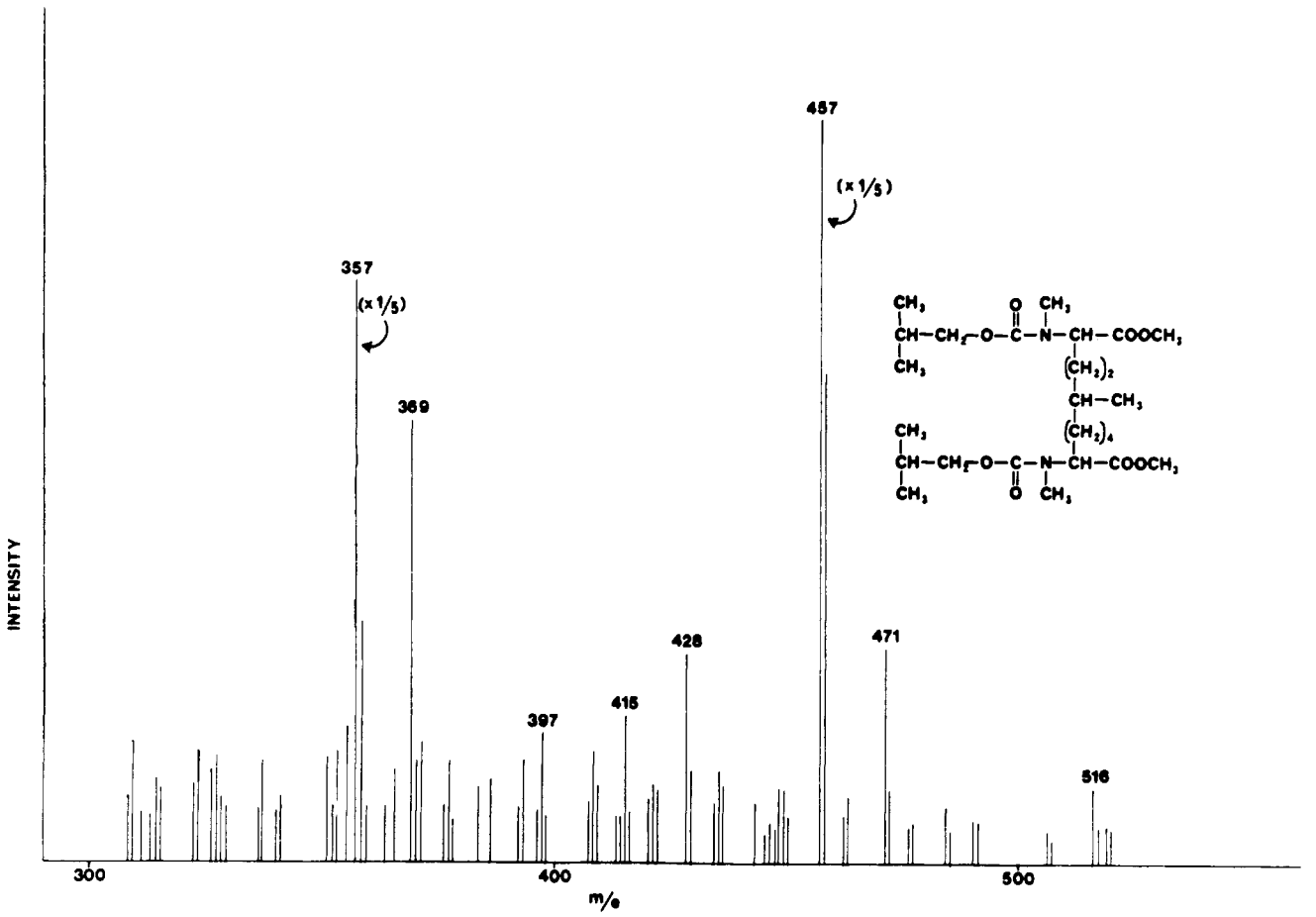

FIGURE 3: Mass spectrum of Iboc-permethylated compound E.

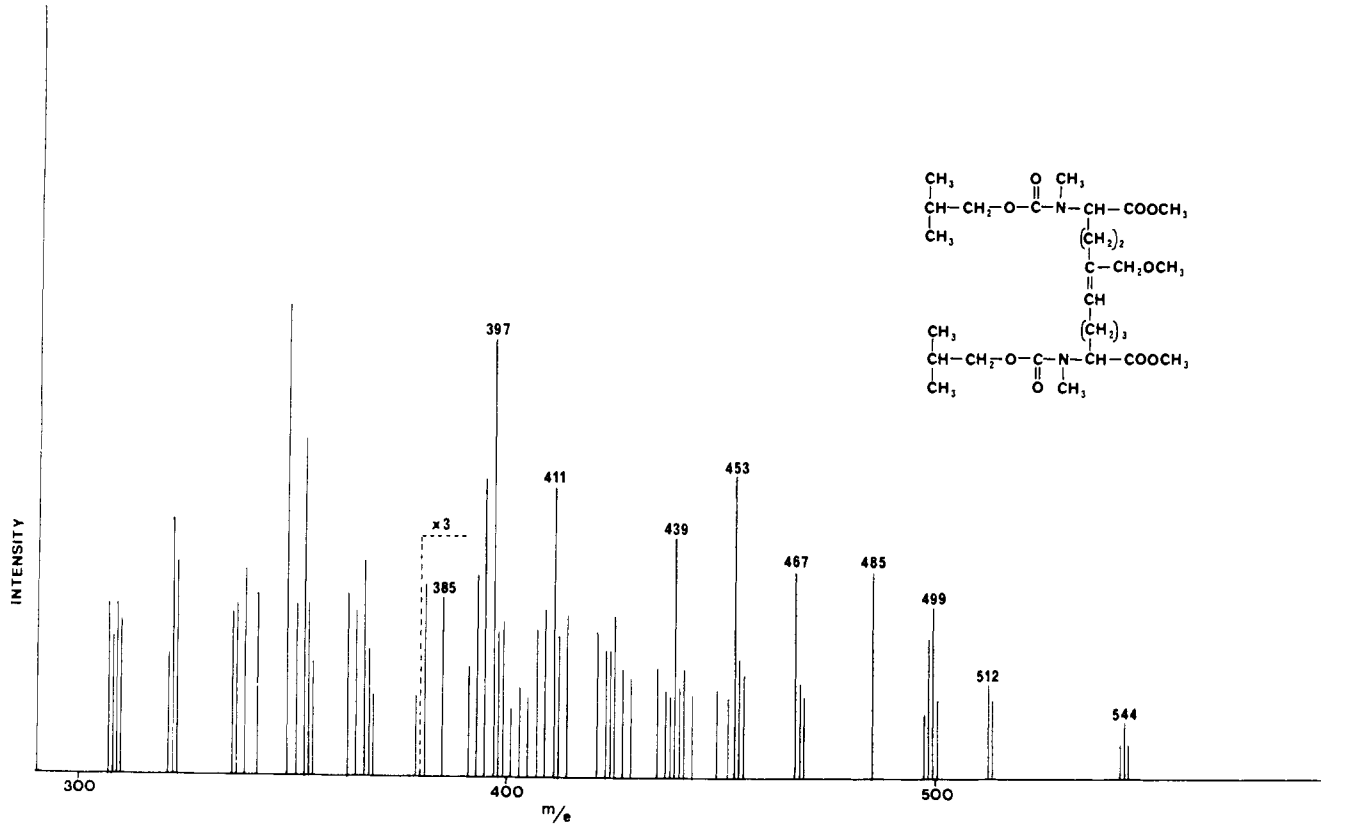

FIGURE 4: Mass spectrum of Iboc-permethylated reduced ACP.

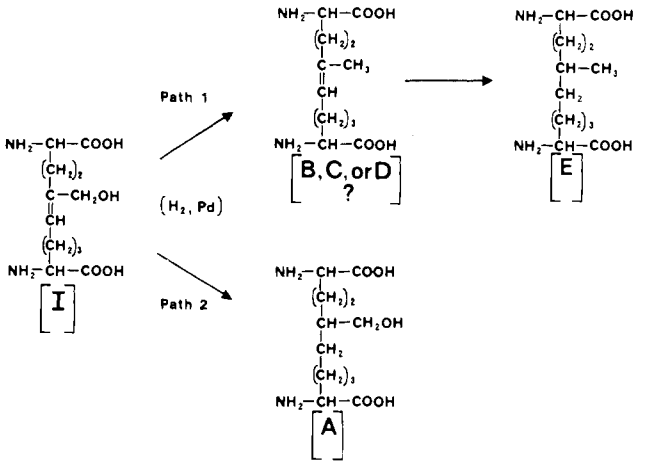

FIGURE 5: Catalytic hydrogenation of reduced ACP.

Mass Spectrometry. Compound A and compound E were derivatized for studies in the mass spectrometer according to the method of Paz *et al.* (1970). Briefly the method involves an initial isobutoxycarbonylation (Iboc) with isobutylchloroformate. This is followed by permethylation of the Iboc derivative employing CH_3I, $(CH_3)_2SO$, and NaH according to the procedure of Hakomori (1964). The mass spectra of such derivatives were obtained from a Hitachi Perkin-Elmer Model RMU-6E mass spectrometer. The mass spectra of derivatized compound A and compound E are given in Figures 2 and 3, respectively. The mass spectrum of the derivatized reduced ACP before hydrogenation is given in Figure 4.

The derivative of the reduced ACP prior to hydrogenation revealed a parent ion of 544. Mass losses of 32 ($-CH_3OH$), 45 ($-CH_2OCH_3$), 59 ($-COOCH_3$), and 91 ($-(COOCH_3 + CH_3OH)$) were all consistent with the original parent ion. The derivative of compound A revealed a parent ion of 546, which would be expected if hydrogenation of the double bond in the reduced ACP (I) had occurred. The most abundant ion corresponded to a mass loss of 59 ($-COOCH_3$). The derivative of compound E showed a parent ion of 516. The most abundant ions were 457, corresponding to a loss of 59 ($-COOCH_3$) and 357 corresponding to a loss of 159. The latter fragment is consistent with the combined radical mass loss of 59 ($-COOCH_3$) and two neutral molecules losses of 56 ($-C_4H_8$) *via* McLafferty rearrangement and 44 ($-CO_2$). This rearrangement of the Iboc group was evident in the mass spectrum of all three compounds examined.

Discussion

Because two separate reactions can occur during the catalytic hydrogenation of an allylic alcohol, the resulting reaction mixture may contain several products. The extent to which one product dominates over the others depends on the amount of catalyst present in the reaction mixture (Doyle and Levenberg, 1968). One of the reactions, hydrogenation, leads only to reduction of the double bond. The second reaction, hydrogenolysis, leads to the replacement of the allylic hydroxyl group with a hydrogen atom, resulting, in the case of reduced ACP (I), in the formation of a methyl group in the place of a hydroxymethyl group. After hydrogenolysis has occurred, the double bond is still available for reduction although such reduction is not obligatory. Accordingly, if an allylic alcohol is subjected to catalytic hydrogenation, one would expect a mixture of products. The major products expected in the case of the reduced ACP are illustrated in Figure 5.

As pointed out by Doyle and Levenberg (1968), catalytic hydrogenation of an allylic alcohol in the presence of large excesses of catalyst leads to extensive hydrogenolysis. It was shown by these authors that L-2-amino-3-hydroxymethyl-3-pentenoic acid yields leucine and alloisoleucine when reduced in the presence of a large excess of PtO (Adams catalyst), indicating that hydrogenolysis followed by hydrogenation of the double bond had occurred. At lower concentrations of catalyst, 2-amino-3-methyl-3-pentenoic acid was detected in addition to the leucine isomers and one other substance. The latter appeared, according to the authors, to be hydroxyleucine. Therefore, at lower concentrations of catalyst several of the possible reaction products were present.

Similar results were obtained in this study. The structures of compound A and compound E resulting from catalytic hydrogenation of reduced ACP (I), are indicated in Figure 5. Compound A is derived from the catalytic reduction of the double bond in the parent compound (I), while compound E is formed by initial hydrogenolysis of the parent compound (I) followed by reduction of the double bond. The results of these studies serve to confirm the existence of an allylic alcohol group within the structure of the reduced ACP isolated from reduced elastin. Compounds B, C, and D were not obtained in large enough quantities to determine their mass spectrum. However, several possible structures for these compounds could be proposed. By analogy to the data of Doyle and Levenberg (1968), one could be the unsaturated compound which would result if a portion of the reduced ACP (I) underwent only hydrogenolysis but not reduction of the double bond (see Figure 5, compound B, C, or D). It should also be pointed out that reduction of the double bond introduces into compound I a new assymetric carbon atom. The resulting isomers might readily be separated on the amino acid analyzer and, this again, could account for another of the unidentified peaks seen on chromatography of the reaction mixture.

The Iboc permethylated derivatives employed in these experiments have proved to be extremely useful for the lysine-derived polyfunctional amino acids, which have been isolated from collagen and elastin. The fragmentation patterns which are obtained from such derivatives are relatively easy to interpret, since these compounds are unable to undergo cyclization to piperidine derivatives upon electron impact excitation in the mass spectrometer. Furthermore, most hydroxylated compounds tend to dehydrate upon excitation making the probability of parent ion detection relatively small. Permethylation, as is well known from carbohydrate chemistry, tends to prevent this from occurring.

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