(items 54–59) differ from *S. alliaria* (item 53) in their lower erucic (13–23%) and higher linolenic acid contents (34–43%). *Lepidium* has four species (items 33, 34, 37 and 38) that have some 10% erucic acid and two (items 35 and 36) that have none. The two species of *Cardamine* are different; one contains dihydroxy acids (item 9) and the other none (item 8).

There was a marked difference in the eicosenoic acid content (27 and 5%) of oils from two species of *Conringia* (items 86 and 87), a parallel difference in linoleic acid (29 and 10%), and an inverse difference in linolenic acid (2 and 44%), but little difference in erucic acid (21 and 26%). The oil of *C. orientalis* analyzed by Hopkins (4,5,7) contain less eicosenoic (about 12%) and more erucic acid (35–40%) than the sample reported here.

Even within a single species there are differences. The seed oil of *Thlaspi arvense* (item 61) reported here has a higher iodine value (148 vs. 122) than that reported earlier (9) and correspondingly higher 18:3 (38 vs. 14%) and lower 22:1 (19 vs. 38%) contents.

Conclusions

Discovery of a unique oil during examination of this relatively small sampling of the family Cruciferae encourages further exploration of the plant world for new chemical composition. Dissimilarity of the two species of *Cardamine* demonstrates the need for examination of all available species rather than for a cursory sampling of families and genera.

The unusual composition of *Cardamine impatiens* oil justifies further study of the properties of the oil and the preparation of derivatives for evaluation in specific end uses. Simultaneously, studies should be initiated to determine where and how to grow the species, and to select or develop strains of improved form and productivity. Similarly, species whose oils are rich in specific acids should be studied to ascertain whether they have advantages in quality of the oil, productivity, or range of adaptability over species now in commercial use.

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In Vitro Inhibition of Lipase Activity by Malonaldehyde, Formaldehyde and Propionaldehyde[1]

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Abstract

The in vitro inhibition of bovine pancreatic lipase by malonaldehyde, formaldehyde and propionaldehyde was investigated. Malonaldehyde, as sodium 3-oxy-prop-2-enal (MA-Na), was found to be the most inhibitory at pH values below 7. Its reaction with lipase appeared to be two part: the first was rapid and a function of the MA-Na concentration; the second part was slower and related linearly to the MA-Na concentration. Methanol-free formaldehyde was a much less effective inhibitor. Low concentrations (0.01 M) had little effect on lipse activity. Propionaldehyde produced the least inhibition. A break point in the reaction of propionaldehyde with lipase occurred with time. After the break point, the inhibition nearly paralleled that seen in the control.

Introduction

REACTIONS OF ALDEHYDES with proteins may be of nutritional and physiological significance in vitro and in vivo. The aldehydes present in a biological system may arise from the autoxidation of lipid materials that produce a diversity of carbonyl compounds. Several autoxidizing food lipid systems have been analyzed by other investigators and the carbonyls present isolated and identified (1, 2). Wyatt and Day (3) in following the changes in the distribution of carbonyls in autoxidizing salmon oil found the shorter chain aldehydes predominant with formaldehyde and propionaldehyde in the highest concentration. Another aldehyde, malonaldehyde (MA), has been shown to be an autoxidation product of methylene-interrupted triene and higher polyunsaturated fatty acids (4) and has been determined (by the 2-thiobarbituric acid method) in significant quantities.

Free MA has been reported as an unstable crystalline monomer by Hüttel (5). Ultraviolet absorption spectral analysis indicate that MA in aqueous solution exists as an enal, 3-hydroxy-prop-2-enal (6), which will undergo internal hydrogen bonding to form a cyclic chelate below pH 4.5 (7). These properties may influence its reactions and make it desirable to compare the reaction of MA with a protein to that of formaldehyde and propionaldehyde with the same protein. All three of these aldehydes can occur through autoxidation.

[1] Technical Paper No. 1859, Oregon Agricultural Experiment Station, Corvallis, Oregon.

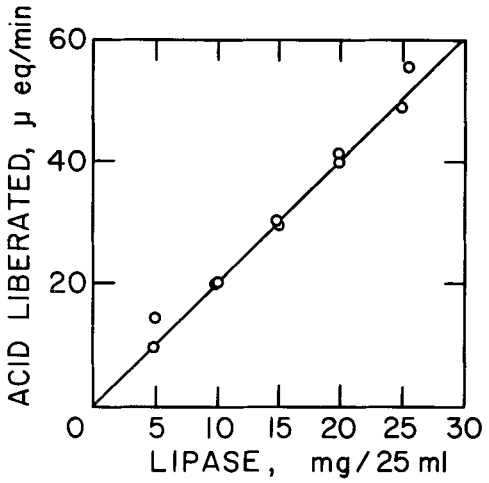

FIG. 1. Relationship between lipase concentration and rate of liberation of fatty acids.

In an extensive study on the effects of formaldehyde on protein, Fraenkel-Conrat and Olcott (8) reported the primary reaction to be methylolamine formation with amines, followed by cross linking with an amide or guanidyl group. Other reactive groups include sulfhydryl, imidazole and indole (9). Recently infrared evidence has been obtained by Milch (10) that aldehydes capable of reacting with proteins exist in aqueous solution in a hydrated form analogous to that of hydrated formaldehyde.

The enzyme lipase has been reported inhibited by formaldehyde, acetaldehyde, butyraldehyde, aldol and benzaldehyde by Weinstein and Wynne (11). Organic peroxides also inhibit lipase according to Wills (12). Monty (13), however, postulated that inhibition of lipase by decylaldehyde when added to the substrate at pH 8.0, was due to alterations of the surface of the emulsion droplets rather than to a reaction of the enzyme.

In this work, malonaldehyde from the autoxidation of polyene fatty acids, is compared with propionaldehyde. The inhibition of the enzyme lipase is used as the basis of comparison. The effects of pH on the aldehyde-lipase reaction and the relative velocities of the reactions are shown.

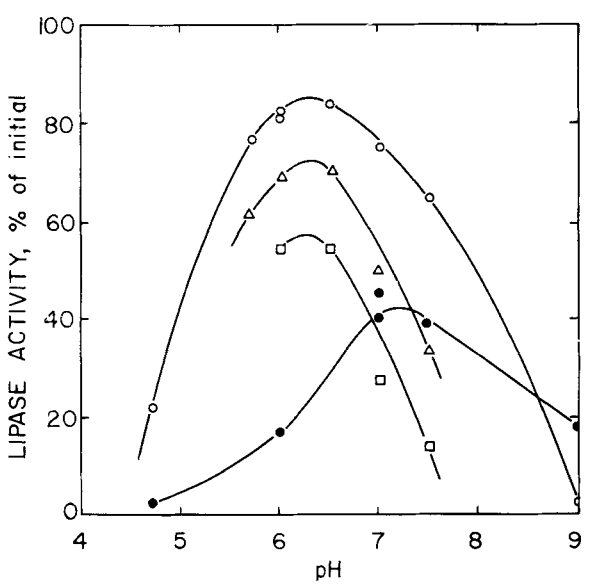

FIG. 2. Effect of pH on lipase stability after 4 hr contact with 0.05 M aldehyde. MA-Na (●), formaldehyde (□), propionaldehyde (△), control (○).

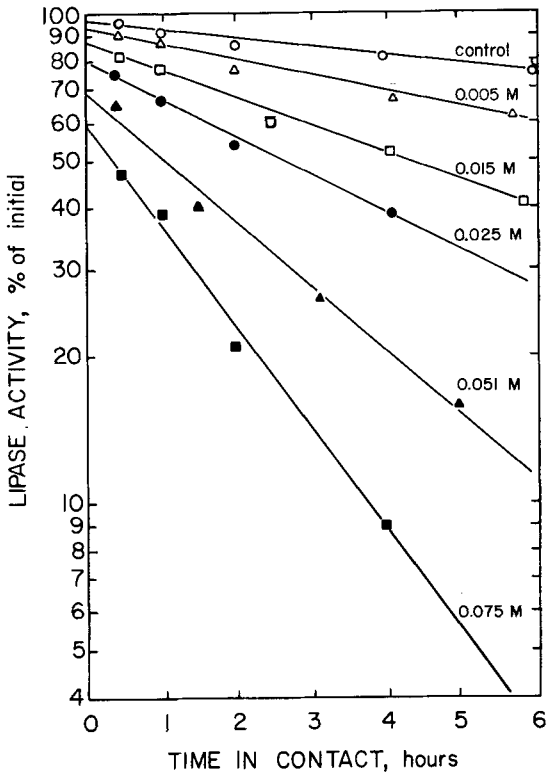

FIG. 3. Lipase inhibition by MA-Na.

Experimental

Bovine pancreatic lipase was obtained from Nutritional Biochemicals Corporation (#448). All enzyme activity analyses were performed at pH 9.0 using the method of Marchis-Mouren et al. (14) with the substitution of 0.1 M sodium taurocholate in place of the 20% solution. This method involved a potentiometric titration of the fatty acids liberated from an olive oil emulsion. A Beckman Zeromatic pH meter was used.

For the stability studies lipase solutions were prepared in cold 0.1 M phosphate buffer at the desired pH. One milliliter aliquots were removed at specific intervals for analysis at 37C. When the enzyme solution was prepared at pH 6.0, the relationship between the release of fatty acids and the amount of enzyme present was found to be linear (Fig. 1). The procedure used to determine the effect of the aldehydes was as follows: the enzyme (0.08% wt/v) was dissolved in cold 0.1 M phosphate buffer at pH 6.0; the aldehyde was added and the solution brought to 25 ml with additional cold buffer. A sample was taken immediately for the initial analysis which served as a reference point for further analyses. The solution was then incubated at 37C with 1 ml portions being removed at 0.5, 1, 2, 4 and 6 hr for analysis.

Malonaldehyde was used as sodium 3-oxy-prop-2-enal (MA-Na), prepared from 1,1,3,3-tetraethoxypropane by the method of Protopova and Skoldinov (15) but by neutralizing at −17C and reprecipitating from aqueous methanol with ether. Methanol-free formaldehyde was prepared from paraformaldehyde by refluxing, and analyzed for formaldehyde content using the sodium sulfite method of Walker (9). For comparison, reagent grade formaldehyde (Merck and Co., Inc., Rahway, N. J.) containing 37% formaldehyde stabilized with 10% methanol was also used. Propionaldehyde, obtained from Eastman Organics, was

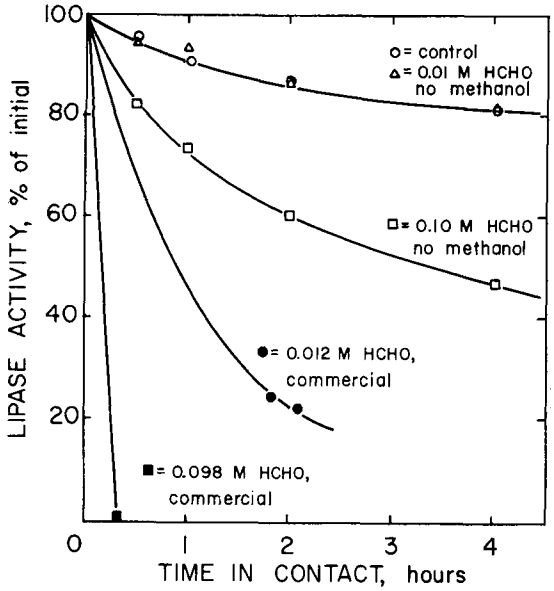

FIG. 4. Effect of formaldehyde with and without methanol on lipase activity.

redistilled and diluted 1 to 50 with distilled water prior to use.

Results and Discussion

Relative Inhibition with pH by the Aldehydes

It would be expected that the reaction of a protein with aldehydes would be dependent upon the particular ionic state of the protein. Thus, at a given pH the aldehyde reactions would be equally affected if they were reacting at the same site. However, the inhibition varied with both the aldehyde and pH (Fig. 2), indicating the reaction was not proceeding in the same manner in all tests. Since methanol-free formaldehyde and propionaldehyde show a different pH inhibitory curve than MA-Na, it is possible that they may be reacting through a different mechanism or with different functional groups. The differences observed may be due in part to the changes which pH can induce in the equilibrium among the forms of malonaldehyde.

Optimum stability of the control after incubation for 4 hr was found to range from pH 6.0 to 6.5 at 37C. At pH 9 the sample containing MA-Na was more stable than the control. This may be inhibition of proteases that contaminate the preparation, or to a direct stabilizing effect. Of the three aldehydes, MA-Na was the most inhibitory at pH values below 7.

Inhibition by MA-Na

The inhibition of lipase by MA-Na may follow first-order kinetics (Fig. 3). The rate constant, k, of the reaction, when plotted against the concentration, indicates a linear relationship between the rate of inhibition and the concentration of MA-Na used. Extrapolating the data in Figure 3 back to zero time shows that the curves do not pass through the origin. The deviation from the origin appears to be a function of the MA-Na concentration; however, this deviation also appears in the control. This deviation indicates a very rapid initial reaction that is followed by a slower reaction. This type of two-stage reaction has been previously discussed by Mihalyi 16) and Fraenkel-Conrat and Olcott (8). It appears that the action of MA-Na is of an irreversible type, characterized by a progressive decrease in activity with time.

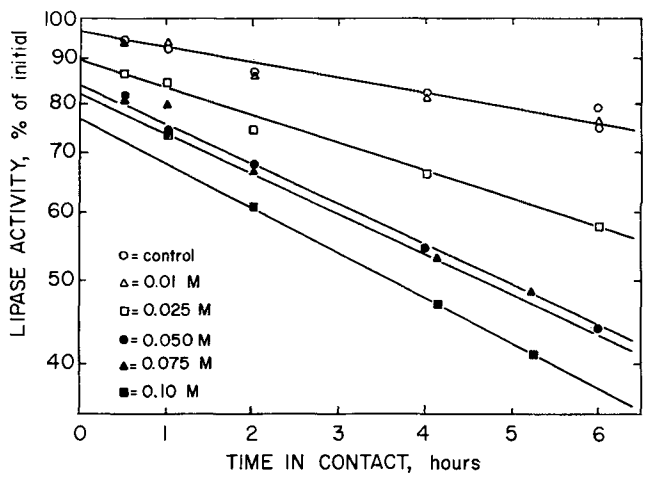

FIG. 5. Lipase inhibition by formaldehyde.

Inhibition by Formaldehyde

Commercial formaldehyde containing 10% methanol was found to be more inhibitory of lipase activity than comparable solutions prepared without methanol from paraformaldehyde (Fig. 4). Methanol-free formaldehyde was used thereafter.

At low concentrations, methanol-free formaldehyde appeared to be much less inhibitory than equal concentrations of MA-Na (Fig. 5). Concentrations of 0.01 M had little effect on lipase activity. At concentrations greater than 0.05 M, increases in concentration did not produce commensurate changes in the degree of inhibition, possibly due to the enzyme nearly reaching a saturation of groups reacting with this aldehyde. The reaction could be two-stage, as with MA-Na, but the data are not as definitive. The reaction may be more ambiguous due to the lower degree of specificity of formaldehyde. The primary reactions of formaldehyde with protein have been given as methylolamine formation by reaction with primary amines followed by methylolamine reaction with amides producing cross links (8).

Inhibition by Propionaldehyde

The action of propionaldehyde on lipase differs from that of both formaldehyde and MA-Na. From Figure 6 it can be seen that the curves of the inactivation of lipase by propionaldehyde passed through the origin, and that the reactions were proceeding at a much

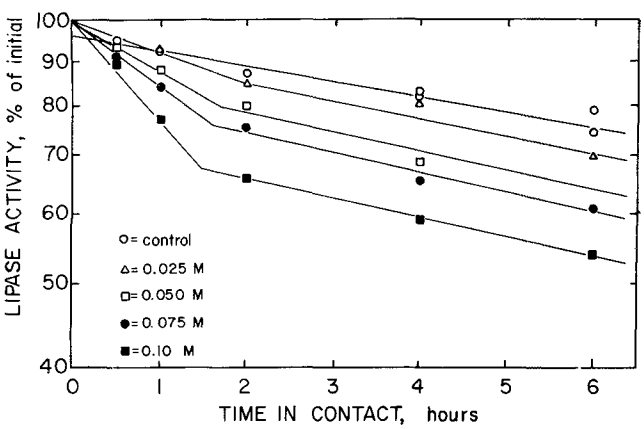

FIG. 6. Lipase inhibition by propionaldehyde.

lower rate. The curves also show a break point that occurs after about 1.5 to 2 hr of reaction. Variations in the propionaldehyde concentration did not change the time needed to reach the change in velocity at the break point. However, the amount inhibited at the break point did depend upon the aldehyde concentration, as did the total inhibition at the end of 6 hr. The early portion of the reaction may represent a reaction of a particular functional group imparting some inhibition, perhaps of asteric nature. The latter portion of the curve, where the lines are nearly parallel, may represent predominantly a continuation of the inhibition due to factors other than any aldehyde present seen in the control. It is evident that the inhibition imparted by propionaldehyde is not as great as that with MA-Na, its dialdehyde counterpart, for any comparable concentration.

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Amines from Aldehydes Derived from the Ozonization of Soybean Esters[1]

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Abstract

Investigations were carried out on reductive amination of caproaldehyde, pelargonaldehyde and azelaaldehydate esters, obtainable from ozonolysis of soybean oil products, with ammonia and hydrogen in the presence of nickel catalyst. A solvent system giving good yields of primary amine while suppressing amide formation was devised. Excess ammonia and homogeneous solutions suppressed secondary amine formation. Nonpolar solvents suppressed ammonolysis. Optimum conditions for reaction varied with the aldehyde.

Excellent yields of hexylamine (91%), nonylamine (90%), methyl 9-aminononanoate (92%) and butyl 9-aminononanoate (93%) were obtained from caproaldehyde, pelargonaldehyde, methyl azelaaldehydate and butyl azelaaldehydate, respectively, when aminated in anhydrous ammonia and either cyclohexane or methyl cyclohexane.

Introduction

THE CLASSICAL METHOD of preparing primary amines by reductive alkylation of ammonia with aldehydes in the presence of hydrogen and a hydrogenation catalyst has been the subject of numerous publications and patents for nearly half a century. Ammonolysis of carboxylic esters with ammonia and amines to form amides is equally familiar. In addition, these routes to amines and amides have been the subject, in part, of at least three reviews (3,5,16). However, little mention is made in these reviews of the N-alkylation of compounds containing both functional groups in the same molecule, such as $OHC(CH_2)_xCOOH$ or $OHC(CH_2)_xCOOR$. We previously reported on the preparation of such bifunctional materials in excellent yield by the ozonization of soybean esters (2,13,14). The excellent yields and potential of such compounds as fiber-forming intermediates prompted us to explore the possibility of devising an economic route to nylon-9 (a fiber-forming polymer exhibiting toughness, wet strength and lack of water absorbency) from unsaturated fatty acids.

Although primary amines can, and have been, prepared in good yield by other methods, such as the reduction of oximes and phenylhydrazones, our investigations were confined to reductive alkylation because it involved but one operation and would therefore be preferable to other methods.

Otsuki and Funahashi (10,11) have described in British and U.S. patents the ozonization of oleic, erucic, ricinoleic and undecylenic acids to their respective ω-formyl acids. In the best example cited, the ozonide of oleic acid was reduced with sodium sulfite and the resulting aldehydic acid reductively aminated in liquid ammonia and absolute alcohol to give a 78% yield of 9-aminononanoic acid. In another example, ethyl azelaaldehydate was prepared and aminated in the same manner as the aldehydic acid to give a 73% yield of ethyl 9-aminononanoate. Likewise, Carpenter and Reeder (4) reductively aminated ethyl azelaaldehydate to give a 51% yield of the amino ester. Pollart and Miller (12) obtained by direct reductive amination of the ozonolysis products of oleic acid a 29% yield of 9-aminononanoic acid and, of methyl oleate, a 52% yield of methyl 9-aminononanoate, with no ammonolysis of the ester reported. However, in another

[1] Presented at the AOCS meeting, Chicago, 1964.
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