Biodegradation of Estolides from Monounsaturated Fatty Acids

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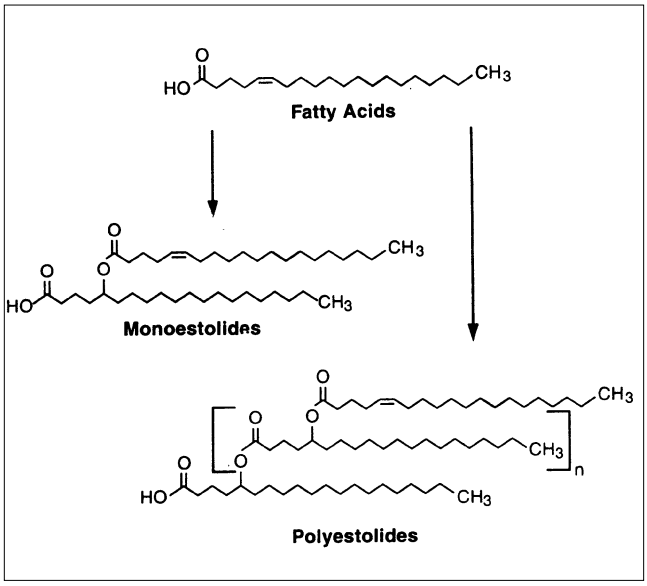
ABSTRACT: Mono- and polyestolides, made from oleic acid, meadowfoam oil fatty acids and erucic acid, were subjected to biodegradation with mixed cultures of *Penicillium verucosum, Mucor racemosus,* and *Enterobacter aerogenes.* Fermentations were continued for 3, 5, 10, 15, 20, or 30 d. Meadowfoam oil and its fatty acids, oleic acid and soybean oil were also biodegraded under the same conditions. After 10 d, oleic acid and soybean oil were degraded 99.8 and 99.2%, respectively; meadowfoam oil and its fatty acids were degraded 89.0 and 97.7%, respectively. After 30 d, oleic acid-derived poly- and monoestolides were degraded 98.6 and 90.0%, respectively, meadowfoam estolides were degraded 75.7%, and erucic acid estolides were degraded 84.0%. *JAOCS 74,* 605–607 (1997).

KEY WORDS: Biodegradation, erucic acid, estolides, meadowfoam oil fatty acids, oleic acid.

Estolides are formed when the carboxylic acid group of one fatty acid forms an ester link at the site of unsaturation of another fatty acid. The structure of polyestolides, containing more than one ester link, and monoestolides, containing one ester link, are shown in Scheme 1. These compounds have a variety of potential applications as lubricants, greases, plastics, inks, cosmetics, and surfactants (1–4). Biodegradability is an advantage for many of these industrial applications. Several tests have been used to determine biodegradability of compounds in nature (5–8). In this study, we tested the biodegradability of estolides by a mixture of microorganisms that are commonly found in nature and are known to utilize vegetable oil-based polymers (6–8). Previous studies have shown that mixtures of microorganisms gave the same results as studies conducted with one microorganism per experiment (7). Testing with this mixture of microorganisms allows the organisms to work together and does not require aeration, which comes close to duplicating the conditions in nature (5). Although the organisms used in these experiments are found in soil, they are equally active in aqueous media.

EXPERIMENTAL PROCEDURES

Materials. Estolides used in this study were prepared from meadowfoam oil fatty acids, oleic acid, and erucic acid (2–3).



SCHEME 1

The extent of their biodegradation was determined by comparing the amount of material that was recovered at termination of the reaction to the amount that was added at the beginning. A mixture of *Penicillium verrucosum* (NRRL 5573), *Mucor racemosus* (NRRL 5281), and *Enterobacter aerogenes* (NRRL B-562), obtained individually from Agriculture Research Service Culture Collection, National Center for Agricultural Utilization Research, were used to test biodegradability. Thiamine hydrochloride, zinc sulfate heptahydrate, magnesium sulfate, phosphoric acid, and dipotassium hydrogen phosphate were purchased from Aldrich Chemical Co. (Milwaukee, WI). Manganese (II) sulfate monohydrate, asparagine, dextrose, iron (II) sulfate heptahydrate, and hexane were purchased from Fisher Scientific Co. (St. Louis, MO).

Methods. A 1. 0-L solution, containing 2.0 g asparagine, 1.0 g dipotassium hydrogen phosphate, 0.5 g magnesium sulfate, 2.0 g dextrose, 5.0 mg thiamine hydrochloride, 1.45 mg iron (II) sulfate heptahydrate, 0.88 mg zinc sulfate heptahydrate and 0.23 mg manganese (II) sulfate monohydrate, was sterilized as a test medium for the biodegradation studies. The three microorganisms mentioned above were individually grown on yeast-malt agar slants and then diluted with 10.0

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mL of sterilized 10% glycerol solution. The diluted microorganisms were combined, and 3 mL of this mixture was added to 1 mL of test substrate and 250 mL of test medium. The flasks were shaken (200 rpm) on a rotary shaker at 28°C for the desired number of days. The fermentations were terminated by refrigeration at 0°C.

The residual lipid material was extracted out of the fermentation broth with hexane and weighed. The efficiency of the hexane extractions was 99.5%, based on extractions of substrate in blank samples, in the absence of microorganisms. Three samples were taken for each data point, and reported results are their averages. Each sample was extracted eight times with fresh hexane (150 mL). The hexane extracts were combined, washed with water (800 mL), and then dried over anhydrous magnesium sulfate. The hexane solutions were then filtered into tared flasks and hexane was removed with a rotary evaporator. Percentage degradation was calculated as 100 × weight of substrate lost/weight substrate added. The extraction efficiency was determined on mixtures that went through the same fermentation cycle in the absence of microorganisms. Each experiment was repeated three times, and the average of the three values is reported.

RESULTS AND DISCUSSION

The first study was made with oils and fatty acids, whose composition is given in Table 1 (2). To determine the effectiveness of the microorganisms, soybean oil, oleic acid, meadowfoam oil, and its fatty acids were tested with a mixture of the above microorganisms (Fig. 1). Oils and fatty acids were degraded 90% or more in 10 d. The longer-chainlength fatty acids in meadowfoam degraded less rapidly. There is also a steric hindrance factor because degradation starts from the carboxylic acid end of the molecule (6). The triglyceride structure is not as accessible to the microorganisms as are free fatty acids. Therefore, free fatty acids degrade faster than oils. However, for meadowfoam oil fatty acids, the chainlength retardation has a larger effect than the steric hindrance effect compared to soybean oil and oleic acid. Soybean oil and oleic acid degrade faster than meadowfoam oil fatty acids. The same effect is seen in estolides (Fig. 2). The longer-chain meadowfoam oil fatty acid estolides and erucic acid estolides degrade slower than the shorter chain oleic acid estolides. The position of the unsaturation in erucic acid helps it biodegrade faster than meadowfoam oil fatty acids because the 13-position in erucic acid is probably more accessible to the microorganisms than the estolide bonds that are mostly on the 5th and 6th carbons in meadowfoam oil fatty acid estolides. Oleic acid polyestolides degrade faster than oleic acid monoestolides, but we have not fully investigated the reasons for this. Degradation of estolides has been compared to the starting fatty acids and triglycerides in Figures 3 and 4. Estolides degrade slower than fatty acids or triglycerides. However, their degradation is rapid enough to be classified as having inherent ultimate biodegradability. Biodegradation tests can be broken down into three groups: (i) Ready biodegradability tests utilize stringent methods, which provide limited opportunity for biodegradation to occur. Compounds that enter this class must biodegrade over 60–70%, depending on the followed procedure, to be considered as readily biodegradable. (ii) Inherent biodegradability tests provide prolonged exposure of the substrate to microorganisms under more favorable

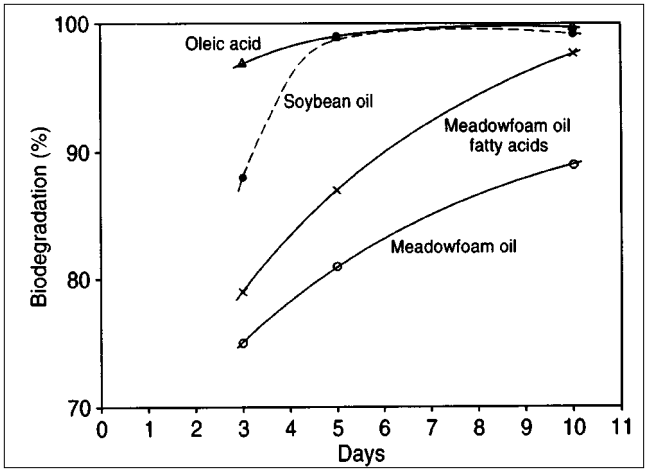

FIG. 1. Biodegradation rates of soybean and meadowfoam oils and their fatty acids.

TABLE 1
Fatty Acid Composition of Oils

	Meadowfoam	Soybean
$<C_{18}$	0.5	10
18:0	—	4
18:1	1.4	22
18:2	0.5	54
18:3	—	7.2
20:0	0.5	—
20:1(Δ5)	64	—
22:1(Δ5)	3	—
22:1(Δ13)	10	—
22:2(Δ5,Δ13)	19	—

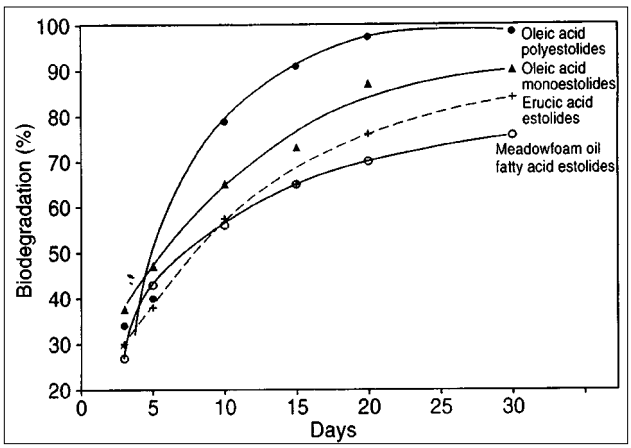

FIG. 2. Biodegradation rates of mono- and polyestolides.

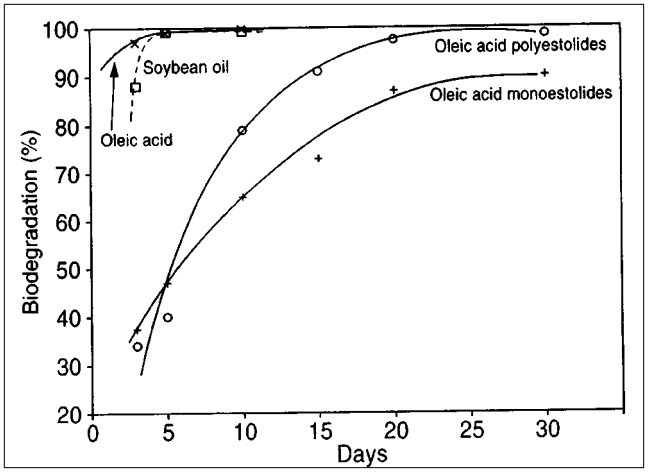

FIG. 3. Biodegradation of oleic acid estolides compared to oleic acid and soybean oil.

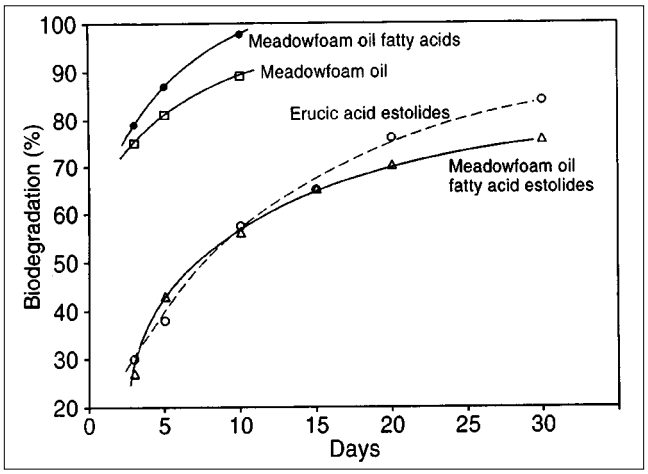

FIG. 4. Biodegradation of meadowfoam oil and erucic acid estolides compared to meadowfoam oil and its fatty acids.

test conditions. These tests are designed to assess if the chemical has potential for biodegradation. Degradation of 20% will be considered as primarily biodegradable, and over 70% is regarded as evidence for ultimate biodegradability. (iii) Simulation tests are run under a specific set of conditions that apply to specific situations (5).

Oleic acid estolides degrade over 90% in 30 d. Oleic polyestolides degrade even faster and pass the 90% degradation level in 15 d. Meadowfoam oil fatty acid estolides are slower to degrade. However, at 30 d, degradation is greater than 75% and is still proceeding with no indication of slowing down. These studies show that, including meadowfoam oil fatty acid estolides, all estolides had good biodegradability and promise rapid and inherent ultimate biodegradability in nature.

ACKNOWLEDGMENTS

The authors express their sincere gratitude to Dr. Stephen W. Peterson for guidance provided in preparation of the microorganisms, to Wanda Brown for her help with inoculation procedures, and to Soyla Nunez for extraction and isolation procedures.

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[Received July 5, 1996; accepted January 24, 1997]