2009

# Synthesis of fatty acid derivatives as potential biolubricants and their physical properties and boundary lubrication performances

Linxing Yao
*Iowa State University*

# Synthesis of fatty acid derivatives as potential biolubricants and their physical properties and boundary lubrication performances

by

Linxing Yao

A dissertation submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Food Science and Technology

Program of Study Committee:
Tong Wang, Major Professor
Earl G. Hammond
Lawrence A. Johnson
Basil J. Nikolau
Philip M. Dixon

Iowa State University

Ames, Iowa

2009

TABLE OF CONTENTS

## ABSTRACT

The desire to replace petroleum-based materials with environmentally friendly and sustainable alternatives has stimulated the development of vegetable oil-based materials as biolubricants. Our studies have focused on molecules that might be produced by biosynthesis of genetically-altered oilseed plants with limited post-harvest modification. Various fatty esters based on oleic acid and ricinoleic acid, were synthesized as potential biolubricants. These include oleate esters of isopropanol, oleyl alcohol and normal alcohols of 1–12 carbons chain lengths; ricinoleate esters of isopropanol and normal alcohols of 1–5 carbons chain lengths; ricinoleate esters acylated in their 12-positions with various acids; oleate esters of ethylene glycol, 1,2-propanediol, 2,3-butanediol, and pentaerythritol. The 12-methyltetradecanoate and decanoate esters of selected polyols were also made. The melting points and viscosities of these esters were documented. Some of these compounds showed melting points and viscosities suitable for uses as biolubricants. 2,3-Butanediol monooleate, butyl ricinoleate and various ricinoleate esters acylated at the 12 positions with short-chain acids were particularly promising. Some of these esters were measured for their boundary lubrication properties with a microtribometer. Seemingly, the lubricities of these esters were affected by their chemical structures. A possible explanation of the lubricity effects based on molecular packing of these compounds on metal surfaces was proposed.

# CHAPTER 1. GENERAL INTRODUCTION

## Rationale

Vegetable oils, such as olive oil, were the dominant lubricants from as early as 1650 BC until the introduction of petroleum based mineral oil in mid-1800s [1]. Later, the industrial revolution led to rapid and impressive development and production of automobiles, which greatly increased the market demand for lubricants. Low- priced mineral oil was more thermally and oxidatively stable than vegetable oils, and it has dominated the lubricant market since then [2,3]. Meanwhile, significant effort was devoted in developing synthetic oils with improved properties. Synthetic oils based only on hydrocarbons were introduced in the 1870s [4]. These "tailor-made' synthetic oils had a superior performance compared to vegetable oil or petroleum-based mineral oil, but they required a premium price. An intensive review on the uses of lubricants in 1970s was prepared by Friedrich [5]. Even with just a small market share, rapeseed oil, crambe oil, castor oil, palm oil, lard, and marine oils found specific application in certain lubrication areas. But the development of lubricants from vegetable oils slowed because of economic and performance factors. Only a few oils, such as rapeseed and high-oleic oil, were used as base oils for lubricants until the early 1990s [6].

The world's demand for petroleum oil has grown dramatically during the past decade whereas the supply of crude oil has grown much more slowly. In the long run, one solution to reducing society's reliance on petroleum is to promote alternative energy technologies. But society will always need lubricants and the development of petroleum substitutes from renewable sources can partly relieve petroleum dependency. Vegetable oil may not be a viable total replacement for fossil fuels in transportation and energy usage because it is not possible to produce enough triglyceride oils to substitute for more than 10% of current petroleum consumption. Currently, over 125 million metric tons per year of vegetable oils are produced worldwide [7], which is about 3% of worldwide consumption of petroleum oil in 2007 [8]. Thus, it would be more appropriate to market oilseed crops in high-value applications such as lubricants and as industrial raw materials [9].

Currently, the costs for raw materials from renewable resources are more comparable with those from petroleum sources due to the rapid depletion of world fossil fuel reserves. The price advantage of petrochemicals disappears as energy costs increase. Price is no longer the main issue preventing the growth of market penetration by bio-based lubricants. Moreover, public desire about a looming petroleum substitutes has triggered a new round of development of bio-based, environmentally friendly lubricants. Bio-based oils are particularly preferred in applications where there are direct contacts with the environment. Hydraulic fluids are major application areas for biodegradable lubricants in the U.S. market. Cutting-and-driving chain oils, two-stroke engine oils, chainsaw-bar oil, marine oils, oil for water and underground pumps, rail-flange lubricants, agricultural equipment lubricants, metal-cutting oils, etc, all have need of environmentally friendly alternatives.

The advantages of lubricants from renewable sources have long been recognized. In general, lubricants based on renewable sources had very low or almost negligible aquatic toxicity and are, in most cases, readily biodegradable [10]. The favorable ecological properties of biolubricants were summarized by Willing [10,11]. Biodegradability mainly depends on chemical structure. Frequently, higher chemical stability, entails reduced degradation rates; however, polyol esters, for example, which have relatively high thermal stabilities among the esters from renewable sources, are still readily biodegradable [11]. Currently, more than 60% of the lubricants used in the United States become pollutants through spillage, evaporation, and total loss lubrication (chainsaw oils, two-stroke engines, concrete mould release oils, exhaust fumes in engines and metal cutting and forming processes) [12,13]. The use of low toxicity, bio-based lubricants would entail fewer hazards and environmental disposals problems.

Bio-based oils often have low volatilities and high viscosity indices (VI) because of their high molecular weights. They have about 20% lower rate of evaporation than mineral oil-based fluids [13]. The viscosity of oil usually increases as temperature decreases and vice versa. The variation of viscosity with temperature is an important parameter in lubrication engineering and is arbitrarily calculated by VI. A narrow range of viscosity change with temperature, i.e. a higher VI, is favored which requires a relatively high viscosity at high temperature and relatively low viscosity at low temperature. This property results in lower oil

consumption and less wear [14]. Ester bonds enable oil molecules to cling to metal surfaces via physical bonding and provide better boundary lubricity than nonpolar petroleum-based mineral oil. Also, bio-based oils have superior compatibility with additive molecules [15].

However, typical vegetable oils, such as soybean or rapeseed oils cannot fully meet the performance criteria for most lubricants. High levels of unsaturated fatty acids, such as oleic, linoleic, and linolenic acids, present in plant oils, and maintain the fluidity of cell membranes. But the presence of bis-allylic protons makes these oils susceptible to oxidation. Increasing the degree of saturation of the oil usually results in poor low-temperature properties. Most of the vegetable oils crystallize when the temperature is below refrigeration temperature. The solidification points of common vegetable oils are summarized in Table 1 [16]. Other shortcomings of vegetable oils include a deposit-forming tendencies, and low-hydrolytic stabilities [15].

**Table 1** Solidification point of common vegetable oils

| Name | Solidification point* (°C) |
|---|---|
| Castor oil | -17 to -18 |
| Corn oil | -10 to -20 |
| Cottonseed oil | 12 to -13 |
| Linseed oil | -19 to -27 |
| Palm oil | 35 to 42 |
| Palm kernel oil | 27 |
| Peanut oil | 3 |
| Rapeseed | -10 |
| Safflower oil | -13 to -18 |
| Sesame oil | -4 to -6 |
| Soybean oil | -10 to -16 |
| Sunflower oil | -17 |

*The solidification points depend on varieties.

The performance limitations of bio-based oils can be overcome by genetic modification of plants, chemical modification of the oil, processing changes, and improved additive technologies [15]. It is expected that fatty acids with desired lubricant properties can be produced in plants by using genetic engineering techniques. So the oil with suitable lubrication properties can be produced after minimal extraction and refining and without further modification. An economic analysis conducted by researchers at Iowa State

University, Ames, IA, showed that biodiesel produced directly in genetically engineered soybeans could successfully compete with petroleum diesel since the former minimizes the cost of refining the vegetable oil and its conversion to methyl esters [17]. This analysis was reported in 2005 and calculations were based on the price of petroleum diesel at $1.5~3.5 per gallon. At present, price of petroleum diesel has increased to twice that in 2005, so the advantages of biodiesel produced from genetically engineered soybeans could be even better if the price of soybeans had remained constant. This prospect also applies to biolubricants produced from genetically engineered oilseeds since lubricants command a much higher price than fuel.

But what types of novel fatty acids derivative might be synthesized in oilseed plants? Do they have desirable properties for lubrication after simple extraction and refining? It is more logical to focus on a few feasible targets rather than trying to introduce a number of radical changes into plants.

In addition, genetically engineering oilseed crops alone might not be capable of providing a sufficient variety and quantity of oil. Combining genetic modification with chemical modification might be necessary to achieve the goal of substituting petroleum-based lubricants with lubricants from renewable sources. Therefore, the objectives of this research are 1) to design and document the properties of biolubricants that might be biosynthesized in oilseed crops while maintaining proper lubrication properties, i.e. low melting point, suitable viscosity, high stability to oxidation, and good lubricity; and 2) to explore the possible feedstocks derived from engineered oilseeds after minimum chemical modifications.

## Literature Review

### Engineering oilseed crops

There are a number of good reviews that extensively summarize the recent developments and achievements of genetically engineering oilseeds and associated problems [9,18-21]. A large variety of fatty acids are synthesized naturally, many of which are

potentially valuable materials or building blocks for industrial uses. However, such fatty acids are either present in insufficient amounts, such as branched chain fatty acids or present in plant species that do not have suitable agronomical characteristics such as ricinoleic acid. Ideal oil for industrial applications would consist of a particular type of fatty acid at a significant percentage, whose fatty acid could be supplied constantly at a competitively low price compared to mineral oil based products [22].

Considerable effort in engineering oilseeds has been directed towards two areas. One is to identify the genes encoding enzymes that are responsible for synthesis of unusual fatty acids, and transfer these genes to existing oilseed crops to produce novel fatty acids for industrial use. The other is to improve the characteristics of existing oil crops with the aid of molecular biology techniques in order to reduce the contents of undesirable seed components, such as ricin toxin in castor bean, or increase the homogeneity of fatty acids in target plant species [9].

One of the earliest attempts in genetically engineering unusual fatty acid in oilseeds was the production of oils with high lauric acid content in rapeseed. Transgenic expression of a laurate-specific acyl-ACP thioesterase gene from the California bay tree to rapeseed [23-25]) led to the accumulation of over 50% laurate in the seed of rapeseed that originally did not have lauric acid in its oil.

Recently the expression of branched-chain fatty acids into plants has been initiated [26]. Some branched-chain fatty acids have suitable low-temperature properties and are very stable to oxidation [27]. But branched fatty acids are not present in most animals or plants in significant quantity. Two bacillus genes were expressed into *Arabidopsis thaliana* to induce branched-chain fatty acid biosynthesis. But the level of expression has not been satisfactory so far [28].

Among vegetable oils, castor oil has attracted much attention as a building block to prepare functional materials. Approximately 85-90% of the fatty acids in castor oil is ricinoleic acid, or 12-hydroxy-*cis*-9-octadecenoic acid. However, castor is not an agronomically suitable crop due to potent toxins and complex allergens in its seeds. Efforts have been placed on removing the undesirable components in the seeds or to expressing ricinoleic acid in other existing oilseed crops that are free of these toxins and allergens. Up to

20% hydroxy fatty acids was produced in *Arabidopsis* through expression of the castor hydroxylase, which hydroxylated oleic acid to ricinoleic acid [29]. Meanwhile, the content of linoleic acid was reduced by 20%.

An example of modifying the characteristics of existing oilseed crop is the development of high-oleic oils. Although high-oleic oils originally were designed for cooking oil, they have industrial uses. By suppressing of the fatty acid desaturase, which is responsible for converting oleic acid into linoleic acid, oleic acid in soybean seed can accumulate to 86% accompanied by reduced linoleic acid [30]. This high percentage cannot yet be achieved by classic breeding. Tests conducted on genetically modified high-oleic soybean oil showed that this oil was much more oxidatively stable than canola oil or chemically modified soybean oil [2]. Even though it is not currently economically feasible because of the price of the seeds, high-oleic oil still would be a great option for biolubricant developers.

However, recent findings suggest that TAG biosynthesis is far more complicated than we have known [20,31,32]. Other than the pathway for TAG biosynthesis described in textbooks, there are at least two alternative acyl-CoA-independent routes and their relative contributions are unknown. Also, introduced exotic fatty acids sometimes accumulate in cell membranes and result in developmental abnormalities [20]. As more attention is given to molecular engineering, more challenges have to be expected. More fundamental research will be required to completely understand lipid metabolism in plant tissues and show how these processes can be modified to optimize vegetable oils for industrial purposes. The goal is to achieve high levels expression of novel fatty acid in transgenic plants and ensure that novel fatty acids do not enter membrane or signaling lipid pools [20].

Chemical modifications --- carboxyl groups

One way to eliminate the negative properties of vegetable oils and improve their performance is through structural modifications. Various chemical modifications to fatty acids that are currently proposed provide us with valuable guidance to the selection of fatty acid derivatives for genetically engineered oilseed crops. So far, most of the modifications

focus on the carboxyl groups (90%), double bonds, and function groups if any (i.e. hydroxyl group, epoxy group) [33].The most important reaction relating to chemical modification of fatty acids is interesterification. The production of biodiesel, for example, is resulted from the interesterification between triglycerides and methanol. A variety of esters including monoesters, diester, polyolesters, etc, can be synthesized by interesterification between acids and alcohols and are catalyzed by acidic or basic catalysts or lipase.

One of the structural drawbacks of triglycerides is their thermal stabilities due to the presence of a hydrogen on the β carbon on the glycerol backbone. In order to improve the thermal stabilities of TAG, polyols, which do not possess the β-hydrogen, were used to replace the glycerol. Thus, no thermal *cis*-elimination would occur and higher decomposition temperature would be required for radical decomposition [34]. Polyesters prepared from polyols, such as neopentyl glycols, trimethylolpropane, and pentaerythritol, with sodium methoxide or *p*-toluenesulfonic acid as catalyst, provide suitable lubricating properties, i.e. high viscosity indices, low pour point temperatures, high flash point, low volatility, and high thermal stability [35, 36]. The acid portion of the polyesters was based on palm oil, palm kernel oil [37], rapeseed oil [38], etc.

Di(carboxylate)esters have been used as synthetic lubricants for many years. Many synthetic oils are hybrids of petroleum and renewable materials. The commercially available diesters are mostly synthesized by esterification of a linear dicarboxylic acid with a branched monofunctional alcohol. The linear hydrocarbon chains of the diester contribute to the good viscosity index, while the branched polar ends give the lubricant a good pour point. The dicarboxylic acid, for example, is from adipic acid, azelaic acid or sebacic acid. The alcohol is from 2-ethylhexanol, isodecanol, or isotridecanol. Azelaic acid can be obtained from oleic acid via chemical processes. Sebacic acid is an oxidation product of castor oil [33].

An example of monoester was the interesterification between triglycerides of soybean oil and a branched-chain alcohol, such as isopropyl. The resulting isopropyl esters had considerably lower crystallization temperature [39].

Chemical modifications --- double bonds

Soybean oil is one of the major oilseed crops and has a comparatively low price. So soybean oil has been a frequent target for chemical modification. Selective hydrogenation has long been used to reduce unsaturation and achieve plasticity for food uses. Lubricants, which have been prepared from partially hydrogenated soybean oil and linseed oil fatty acids, were used as substitutes for sperm whale oil after slaughtering whales became prohibited in 1969 [6]. In the early 1990's, studies showed that polyglycol derivatives of partially hydrogenated soybean oil had comparable viscosities, flash points and fire points to the feedstocks [6]. These glycol derivatives were suggested for use as antifriction additives, water-based cutting fluids, etc.

Elimination of *cis* double bonds will not only increase the stability of the fatty acids, but also results in an increased melting point. Quite a few studies were conducted to introduce various lengths of branches at the bis-allylic site when double bonds were removed. The resulting esters had a considerable change in properties compared to the starting materials. The double bonds can be easily epoxidized with hydrogen peroxide to form epoxy rings [40]. Epoxidized soybean oil is available in large volumes at a reasonable cost. An epoxy group is readily functionalized by further reactions. After epoxy ring opening, there are several options for making derivatives depending on the acyl donors that are present. An ether or ester group can be added leaving none or one free hydroxy group. Thus, one or two branches with various chain lengths can be added in the middle of the linear carbon chain of the precursor. Improved low temperature and oxidation stability were observed for these esters derived from epoxidized oil [7,41,42]. Their pour points ranged from 6 to -9°C without additives. Incorporation of sulfur was achieved by the synthesis of hydroxy thio-ether of soybean oil from expoidized oil and organic thiols [43].

Chemical modifications --- polymerizations

The development of biolubricants based on estolides derivatives from castor oil, lesquerella oil, and meadowfoam oil [44-46] also have been studied. Estolides are formed by esterification between the carboxyl group of a fatty acid and the hydroxy group of a hydroxy fatty acid. A small amount of estolides has been found to exist in plants [47]. Estolides could

be obtained from chemical reaction under high temperature and low pressure or with strong inorganic acid catalysts [44, 48] or from lipase catalyzed reactions [49]. Estolide formation of hydroxy fatty acids ends with a polymer containing a free hydroxy group on one end. Most studies have suggested that the free hydroxy group should be capped with a non-hydroxy fatty acid that was either saturated or branched. Such polymerization increased the molecular weight of the product while resulting in excellent low-temperature properties. Estolides esters had pour points ranging from -54 to 6°C with various "caps". Estolides triglycerides with "caps" had pour points ranging from -36 to 9°C. These estolides were reported to have suitable viscosities and oxidative stability [44, 50]

Other than these most commonly proposed processes, a number of fatty acid and derivatives are still in the investigation stage, and their properties, performance, and cost are being evaluated. A detailed review has been given by Willing [11].

**Dissertation Organization**

This dissertation contains a general introduction, including a research rationale and a literature review, followed by four research papers and a general conclusion. The titles of the papers are "Melting points and viscosities of fatty acid esters that are potential targets for engineered oilseed", "Synthesis of ricinoleate esters as potential biolubricants and their physical properties", "Melting points and viscosities of various diol and polyol esters as potential biolubricants", and "Boundary lubrication properties of fatty acid derivatives as potential biolubricants", respectively. The papers are in the required corresponding journal formats.

**References**

1. Polishuk AT (1974) Current trends in lubricating greases. NLGI Spokesman 39:126-135 (1974)

2. Scott F (2003) One slick trick: building a better biolubricant. Environ Health Perspect 111:A654-657

3. Lal K, Carrick V (1994) Performance testing of lubricants based on high oleic vegetable oils. Synth Lub 11: 189-206

4. Gunderson RC, Hart AW (1962) Synthetic Lubricants, Reinhold, New York.

5. Friedrich JP (1979) Lubricants. In Fatty Acids, Pryde EH (ed) AOCS press, Champaign, IL pp 591

6. Basu HN, Robley EM, Norris (1994) Preparation of glycol derivatives of partially hydrogenated soybean oil fatty acids and their potential as lubricants. J Am Oil Chem Soc 71: 1227-1230

7. Sharma BK, Adhvaryu A, Liu Z, Erhan SZ (2006) Chemical modification of vegetable oils for lubricant applications. J Am Oil Chem Soc 83: 129-136

8. US Department of Energy, Energy Information Administration. May 2008 International Petroleum Monthly, Table 2.4 World Petroleum (oil) Demand, 2003-2007

9. Smith MA (2007) Engineering oilseed for industry. In Issues in New Crops and New Uses. Janick J, Whipkey A (eds) ASHS Press, VA, pp 90

10. Willing A (2001) Lubricants based on renewable resources-an environmentally compatible alternative to mineral oil products. Chemosphere 43: 89-98

11. Willing A (1999) Oleochemical esters-environmentally compatible raw materials for oils and lubricants from renewable resources. Fett/Lipid 101:192-198

12. Erhan SZ, Sharma BK, Perez JM (2006) Oxidation and low temperature stability of vegetable oil-based lubricants. Ind Crops Prod 24: 292-299

13. Schneider MP (2006) Plant-oil-based lubricants and hydraulic fluids. J Sci Food Agric 86: 1769-1780

14. Gunstone FD, Alander J, Erhan SZ, Sharma BK, McKeon TA and Lin JT (2007) Nonfood uses of oils and fats. In: The Lipid Handbook. Gunstone FD, Harwood JL, Dijkstra AJ (eds) Chapman & Hall, London, pp 615

15. Erhan SZ, Adhvaryu A (2002) Vegetable oil-based base stocks. In Biobased Industrial Fluids and Lubricants, Erhan SZ, Perez JM (eds). AOCS press and the Soy Protein Council. pp 1-19

16. Dean JA (1979) Lange's Handbook of Chemistry, 12$^{th}$ ed, McGraw-Hill Inc.

17. Dowling A, Zakharenko O (2005) Assessing biodiesel's cold-flow limitation and gains from re-engineered soybeans. Data not published.

18. Napier JA (2007) The production of unusual fatty acids in transgenic plants. Annu Rev Plant Biol 58:295-319

19. Dyer JM, Mullen RT (2005) Development and potential of genetically engineered oilseeds. Seed Sci Res 15:255-267

20. Murphy DJ (2002) Biotechnology and the improvement of oil crops-genes, dreams and realities. Phytochem Rev 1: 67-77

21. Jaworski J, Cahoon EB (2003) Industrial oils from transgenic plants. Curr Opin Plant Biol 6: 178-184

22. Töpfer R, Martini N, Schell J (1995) Modification of plant lipid synthesis. Science 268: 681-686

23. Voelker TA, Worrell AC, Anderson L, Bleibaum J, Fan C, Hawkins DJ, Radke SE & Davies HM (1992) Fatty acid biosynthesis redirected to medium chains in transgenic oilseed plants. Science 257: 72–73

24. Wiberg E, Dehesh K and Stymne S (2000) The distribution of caprylate, caprate and laurate in lipids from the developing seeds of transgenic oil-seed rape (Brassica napus L.). Planta, 212: 33–40

25. Dyer JM, Stymne S, Green AG, Carlsson AS (2008) High-value oils from plants. Plant J 54: 640–655

26. Nikolau B, Wurtele E, Wang T, Sundararajan S, Hammond E, Jin Y, McMullen K, Yao L, Yu X, Bhuyan S (2005). The use of branched chain fatty acids and branched chain fatty acid biosynthetic genes to alter the thermal, physical and lubrication properties of lipid-based biofuels and biolubricants. Invention Disclosure, ISURF # 03386

27. Yao L, Hammond EG (2006) Isolation and melting properties of branched fatty esters from lanolin. J Am Oil Chem Soc 83: 547–552

28. Personal communciation with Dr. Eve Wurtele

29. Grushcom J, Smith M (2006) Engineering high-performance biolubricants in crop plants. Ind Biotech 2: 48-50

30. Kinney AJ (1996) Designer oils for better nutrition. Nature Biotech 14: 946

31. Dahlqvist A, Stahl U, Lenman M, Banas A, Lee M, Sandager L, Ronne H, Stymne S (2000) Phospholipid: diacylglycerol acyltransferase: An enzyme that catalyses the acyl–CoA-independent formation of triacylglycerol in yeast and plants. Proc. Natl. Acad. Sci. 97: 6487–6492

32. Stobart K, Mancha M, Lenman M, Dahlqvist A, Stymne S (1997) Triacylglycerols are synthesised and utilised by transacylation reactions in microsomal preparations of developing safflower (*Carthamus tinctorius* L) seeds. Planta 203: 58–66
33. Wagner H, Luther R, Mang T (2001) Lubricant base fluids based on renewable raw materials. Their catalytic manufacture and modification. Appl Catal A: General 221: 429-442

34. Eychenne, V, Mouloungui Z, Gaset A (1996) Les lubrifiants à base d'esters de néopentylpolyols, O.C.L. 3: 57–63

35. Eychenne V, Mouloungui, Z (1998) Relationships between structure and lubricating properties of neopentylpolyol esters. Ind Eng Chem Res 37: 4835-4843

36. Mattei L, Pacor P, Piccone A (1995) Oils with low environmental impact for modern combustion engines. Synth Lubr 26: 49-52

37. Yunus R, Fakhru'I-Razi A, Ooi TL, Iyuke SE (2004) Lubrication properties of trimethylolpropane esters based on palm oil and palm kernel oils. Eru J Lipid Sci Technol 106: 52-60

38. Uosukainen E, Linko Y, Lämsä M, Tervakangas T, Linko P (1998) Tranesterification of trimethylolpropane and rapeseed oil methyl ester to environmentally acceptable lubricants. J Am Oil Chem Soc 75: 1557-1563

39. Lee I, Johnson LA, Hammond EG (1995) Use of branched-chain esters to reduce the crystallization temperature of biodiesel. J Am Oil Chem Soc 72: 1155-1160

40. Swern D, Billen GN, Findley TW, Scanlan JT (1945) Hydroxylation of monounsaturated fatty materials with hydrogen peroxide. J Am Chem Soc 67: 1786-1789

41. Hwang H, Erhan SZ (2001) Modification of expoxidiezed soybean oil for lubricant formulations with improved oxidative stability and low pour point. J Am Oil Chem Soc 78: 1179-1184

42. Moser BR, Sharma BK, Doll KM, Erhan SZ (2007) Diesters from oleic acid: synthesis, low temperature properties, and oxidation stability. J Am Oil Chem Soc 84: 675-680

43. Sharma BK, Adhvaryu A, Erhan SZ (2006) Synthesis of hydroxy thio-ether derivatives of vegetable oil. J Agric Food Chem 54: 9866-9872

44. Isbell TA, Lowery BA, DeKeyser SS, Winchell ML, Cermak SC (2006) Physical properties of triglyceride estolides from lesquerella and castor oils. Ind Crops Prod 23: 256-263

45. Cermak SC, Brandon KB, Isbell TA (2006) Synthesis and physical properties of estolides from lesquerella and castor fatty acid esters. Ind Crops Prod 23: 54-64

46. Erhan SM, Kleiman R, Isbell TA (1993) Estolides from meadowfoam oil fatty acids and other monounsaturated fatty acids. J Am Oil Chem Soc 70: 461-465

47. Fehling E (1995) Analysis of estolides in technical hydroxylated fatty acids from plant oils. J Am Oil Chem Soc 72: 355-359

48. Cermak SC, Isbell TA (2001) Synthesis of estolides from oleic and saturated fatty acids. J Am Oil Chem Soc 78: 557- 565

49. Hayes DG, Kleiman R (1995) Lipase-catalyzed synthesis and properties of estolides and their esters. J Am Oil Chem Soc 72: 1309-1316

50. Cermak SC, Biresaw G, Isbell TA (2008) Comparison of a New Estolide Oxidative Stability Package. J Am Oil Chem Soc 85: 879-885

# CHAPTER 2. MELTING POINTS AND VISCOSITIES OF FATTY ACID ESTERS THAT ARE POTENTIAL TARGETS FOR ENGINEERED OILSEED

Modified from a paper to be published in
*The Journal of American Oil Chemists' Society*

Linxing Yao[2,3], Earl G. Hammond[2,4], Tong Wang[2]

**Abstract** Our previous isolation of branched-chain fatty acid (BCFA) methyl esters from lanolin was improved and scaled up to gram-quantities. Also, oleate esters of isopropanol, oleyl alcohol and normal alcohols of 1–12 carbons chain lengths were prepared. Esters were made by interesterification with sodium alcoholates and by esterification with *Candida antarctica* lipase. It proved easier to obtain pure esters by the enzymatic synthesis. Melting points and viscosities over the range of 0–70°C were determined in order to better identify potential lubricant targets that might be produced by genetically modified oilseed crops. Isopropyl and butyl oleate have melting points of -33 and -32°C, respectively, and viscosities that range from ~17 cp (0°C) to ~2.5 cp (70°C). They should have suitable stabilities for lubrication applications. BCFA esters had viscosities similar to their straight-chain analogs. Viscosities increased with alcohol chain length and decreased with temperature. The dependence of viscosity on temperature was fit with an equation based on Erying's rate equation. Some esters with branched acid or branched alcohol moieties, and some oleate esters might be utilized as biolubricants or biofuels on the basis of their melting points and viscosities.

**Keywords** Branched chain fatty esters · Melting point · Oleate esters · Viscosity

---

[1]Reprinted with permission of J Am Oil Chem Soc, 2008, 85, 77-82.
[2]Graduate student, University Professor Emeritus, and Associate Professor, respectively, Department of Food Science and Human Nutrition, Iowa State University.
[3]Primary researcher and author.
[4]Author for correspondence.

## Introduction

Lipids used as lubricants should have suitable viscosities, low melting points and good stabilities to oxidation. For maximum economic advantage they should require only extraction and limited refining to be ready for use. Low-melting lipids with good oxidative stability can be achieved by using esters of monounsaturated fatty acids. An alternate solution is to use branched-chain fatty acids (BCFAs) and alcohols. By expressing surface coat genes that control wax synthesis in oilseed tissue the biosynthesis of such esters might be achieved. Physical property data for such esters is limited, which prevents rational target selection. Previously, we reported the melting points of methyl and isopropyl esters of BCFAs with chain lengths of 13–18 [1]. Their attractive melting points encouraged us to measure the viscosities of these molecules, so we scaled up and refined our previous methods to produce larger quantities of some branched esters from lanolin. Methyl oleate melts at -20°C and has fairly good oxidative stability [2], which suggests that oleate esters might be suitable candidates for biolubricants. In this study, we studied the effect on melting points and viscosities of oleate esters with alcohols of various chain lengths and branching.

## Materials and Methods

### Materials

Lanolin acids were purchased from Rita Corp. (Crystal Lake, IL, USA) with the brand name-RITALAFA. Silica gel (40–140 Mesh) was from J.T.Baker (Phillipsburg, NJ, USA). Lipase acrylic resin from *Candida antarctica* (EC 3.1.1.3), methyl oleate, oleic acid and alcohols of various chain lengths, methyl pentadecanoate and methyl palmitate were purchased from Sigma-Aldrich (St Louis, MO, USA). Aminopropyl extract-clean cartridges and silica gel G Uniplates (250 lm) with inorganic binder for thin layer chromatography (TLC) were purchased from Alltech (State College, PA, USA).

Methods

*Bench-top Scale-up Procedure for Isolation and Purification of BCFAs*

Methyl esters of lanolin free fatty acids (FFA) were made by using Downing's procedure [3]. Lanolin FFA was mixed with benzene, methanol and concentrated sulfuric acid in a ratio of 5:50:50:1 (g:ml:ml:ml), and refluxed for 4–5 h. The benzene solution was extracted once with 2% sodium bicarbonate solution and twice with water. The intermediate emulsion phase was separated by centrifugation, and the benzene solution was filtered through a Buchner funnel containing sodium sulfate. The benzene was evaporated, and residual benzene was removed with a stream of nitrogen gas. The crude FAME was then distilled at 0.5 Torr pressure in a simple insulated still, and the distillate was collected up to a 160°C vapor temperature using an air condenser to cool the distillate. The distillate was further fractionated by chain length in a spinning-band column as described previously [1]. Urea counter-current distribution of methyl esters was used to separate branched, normal and hydroxy compounds with similar boiling points according to their ease of forming urea complexes [1]. Since ~3 g urea is required to complex 1 g long-chain FAME, we added 6 g FAME obtained from a spinning band distillation fraction, to a 500-ml filter flask (designated flask #1) and then added 12–15 g urea and 200 ml of methanol 50%-saturated with urea. Then the mixture in flask #1 was heated to dissolve the FAME and urea and slowly cooled it to room temperature for crystallization. A series of 500-ml filter flasks, each containing 7 g urea, were prepared. A filter stick was used to transfer the liquid from flask #1 to flask #2. Another portion of 200 ml 50%-saturated urea–methanol solution was added to flask #1. The products in flask #1 and #2 were both heated to dissolve reactants and then cooled to crystallize. This process was repeated until 15–18 flasks were filled with the complex. The FAME was released by heating the mixture at 60°C, and then extracted with water and hexane.

*Chemical Synthesis and Purification of Oleate Esters*

Methyl oleate (99% pure) was transesterified with an excess of the desired alcohol (1:200 molar ratio) for about 1.5 h at room temperature. For alcohols with chain length longer than 10, the reactants were heated enough to keep them in liquid state. The alkali catalyst was generated by reacting sodium metal with the various alcohols before the methyl oleate was added. The alcohols were freed of carbon dioxide before adding the sodium by sparging with nitrogen or with reduced pressure. Catalyst was used at ~0.19 meq/g of FAME [4]. After the reaction, adequate acetic acid was added to neutralize the catalyst. The esters were dissolved in hexane and washed with 2% aqueous sodium bicarbonate solution until the acetic acid was removed, usually three times. Residual alcohols that had carbon chain lengths less than 5 could be removed by evaporation under dry nitrogen gas or with a rotary evaporator. Longer chain alcohols were mostly removed by distillation in a water bath at ~100°C and at ~0.1 Torr. Residual alcohol, usually 1–2 ml, was removed by passage through a silica-gel column (1 g silica/0.1 g reaction mixture) with hexane–diethyl ester (90:10 v/v).

*Enzymatic Synthesis and Purification of Oleate Esters*

When doing transesterifications with large excess of the alcohol, it was not economic to remove the residual alcohol with a silica-gel column. To avoid this problem and improve the purities of products, enzymatic syntheses were performed with oleic acid and alcohol at a 1:2 molar ratio in a solvent-free system. *Candida antarctica* lipase was added at 2% by weight. Molecular sieve in an amount, which was calculated to absorb the water generated in the reaction, was added at a ratio of 1 g molecular sieve to 1.8 g water at 3 and 24 h after the reaction started. The reaction took place at room temperature (~25°C) with constant stirring and was stopped after 4 days with the addition of water. The product was centrifuged to remove the enzyme and molecular sieve, and then extracted with hexane and 2% sodium bicarbonate solution. Since alcohols were added in smaller excess than in the chemical syntheses, silica-gel chromatography was applied to the product after extraction to remove residual alcohol and FFA. If subsequent gas chromatography (GC) or TLC detected FFA, these samples were further purified by passing through an amino cartridge with hexane, and the neutral esters were eluted with chloroform-isopropanol (2:1 v/v).

*GC, TLC and NMR*

The purities of oleate esters and BCFA esters were examined by GC. Esters in hexane solution were injected into a HP 5890 Series II instrument (Hewlett-Packer, PA, USA) with a SP-2330 fused silica column (15 m x 0.25 mm and a 0.20 μm film thickness) (Supelco, Bellefonte, PA, USA). The injector and flame detector were at 220°C, and the oven temperature was programmed from 100 to 220°C at 5°C /min. Free fatty acid and residual alcohol also were detected by TLC on silica plates. Hexane–diethyl ether–acetic acid (80:20:1) was used as developing solvent. The separation was visualized under ultraviolet light after spraying with 0.1% (w/v) 20, 70-dichlorofluorescein in methanol. The structures of the oleate esters in $CDCl_3$ were confirmed by $^{13}C$ nuclear magnetic resonance (NMR) with a Varian VXR-400 NMR spectrometer (Varian Inc., Palo Alto, CA, USA).

*Melting Points*

Melting points of the oleate esters were measured with a DSC7 differential scanning calorimeter equipped with an Intracooler System I (Perkin Elmer, Norwalk, CT, USA). Indium and n-decane were used for calibration. The melting onset temperatures of standards were calibrated with melting points listed in the DSC7 operation manual (Perkin Elmer, Norwalk, CT, USA). Methyl stearate was used as a secondary standard, of which the onset melting temperature ($T_{onset}$), peak temperature ($T_p$), and completion of melting ($T_{com}$) are 38.9, 41.9 and 43.1°C, respectively. About 3– 4 mg of each sample was weighed to 0.01 mg in an alumina pan, and an empty pan was used as a reference. The thermal program was in accordance with our previous study and AOCS recommendations [1, 5]. After equilibration at 25°C for 1 min, the sample was rapidly heated to 80°C at 40°C /min and held for 10 min for complete melting of crystal nuclei, cooled to -60°C at 10°C /min rate and equilibrated for 20 min. When reheated at 5 °C /min, $T_{onset}$, $T_p$, and $T_{com}$ were recorded. Each sample was measured at least twice and the DSC instrument had high reproducibility.

*Viscosity*

The viscosities of the various oleate esters and BCFA esters were determined with a Brookfield DV II+ viscometer (Brookfield Inc., Stoughton, MA, USA) using a CP42 cone spindle. The cup was filled with 1 ± 0.03 ml of ester. Temperature was controlled with an Isotemp 3016 refrigerated circulator (Fisher Scientific, Pittsburgh, PA, USA). Temperature was monitored with a certified partial immersion Fisherbrand thermometer (Fisher Scientific, Pittsburgh, PA, USA) with a range of -1 to 101°C calibrated in 0.1°C divisions, which was inserted in a tube on the return line of the circulating water bath. Air pressure was used to keep the water level in the tube at the proper immersion level of the thermometer. Data was collected between 0 and 70°C at 5 °C intervals. Viscosities were recorded after equilibrating at the desired temperature for 10 min and at the maximum possible torque. The viscosity accuracy and repeatability are ±0.2% and ±1.0%, respectively.

**Results and Discussion**

Bench-top Scale-up Procedure

The yield of crude methyl ester from the commercial lanolin FFA was quantitative based on a MW of 256 for lanolin FFA and 270 for FAME. They had nearly the same FAME composition by GC as those of our previous research [1]. The preliminary distillation removed longer chain compounds as well as small amounts of sterol esters. The distillate was rich in FAMEs with chain lengths shorter than 21 C and the distillation pot residual was rich in FAMEs with chain length longer than 18 C. Since no new GC peaks were observed from the distillate or residual, we concluded that distillation did not bring about any undesired reaction. The distillate amounted to ~38.2% of the crude FAME by weight. From the crude distillations, 374 g was collected and fractionated through the more efficient spinning-band column.

The scaled-up counter-current urea separation of the spinning band fractions yielded gram quantities of methyl 12-methyltetradecanoate (15a) and methyl 14-methylpentadecanoate (16i) with 95% purity. The urea separation also removed the hydroxy

FAME and avoided the expensive alumina chromatographic purification step used in our previous study [1].

Oleate Esters Synthesis

Oleate esters of isopropanol, oleyl alcohol and normal alcohols of 1–12 carbons chain lengths were synthesized with enzyme as well as with sodium alcoholate catalyst. The yields of oleate esters by chemical transesterification averaged 85%, and some batches were as high as 94%. The purities of the esters by GC were ~95%. The major impurity always was methyl oleate. Enzymatic syntheses gave yields of ~75% but improved the product's purity to 99% since the excess oleic acid was easily removed from the nonpolar oleate esters with an amino cartridge or TLC. The $^{13}C$ NMR chemical shifts of oleate esters were assigned to various carbon atoms by comparing observed values with calculated values obtained by ACD/ChemSketch Predictor (Advanced Chemistry Development Inc., Toronto, ON, Canada) and Vieville et al [6] result. The $^{13}C$ NMR spectra of the oleate esters all showed carbonyl groups at 174.4–175.3; the methylene adjacent to the carbonyl group at 61.5–66.6, except for the isopropyl at 68.3; the methylene β to the carbonyl at 35.1–35.8, double bond carbons at 130.3–131.3; terminal methyl groups at 11.2–15.4 except for the isopropyl ester at 22.9; a carbon α to the terminal carbon at 14.7–15.6, a carbon β to the terminal carbon at 23.2–24.1; a methylene carbon adjacent to the ester bond at 61.5–66.6 for the normal chains and 68.4 for the isopropyl ester; other methylene carbons ranged from 23.7 to 33.3. In most instances, the extreme values for the straight-chain esters belong to the ethyl, propyl or butyl ester values, which is undoubtedly a reflection of the terminal groups of the alcohols.

Melting Point

The esters from enzymatic synthesis were used for melting point measurements since they had higher purities than those of alkali-catalyzed reaction. The melting points of oleate esters are shown in Table 1. Of all the esters examined, the lowest melting point was that of isopropyl oleate at -33.0°C. Among even-chain normal alcohols, butyl oleate melted lowest

at -31.7°C; and among the odd chains, propyl oleate was lowest at -27.2°C. Pentyl and hexyl oleate's melting points were very close at -25.2 and -25.8°C, respectively. The equipment manufacture's precision claim is better than ±0.1%, and temperature accuracy is ±0.1°C; however, the AOCS has reported that the standard deviation of determination among laboratories was ±1.8°C for the onset temperature and 0.8°C for the completion [5]. The melting points of methyl through pentyl oleate agreed with the results of Candy et al. [7]. Plots of the melting points of the straight-chain ester versus the number of carbon atoms in the alcohol moiety showed a decreasing melting point until carbon number four, and then an increasing value (Fig. 1). Literature values of corresponding esters of various saturated fatty acids as well as FFAs show similar melting patterns, but at considerably higher temperatures than the oleate esters [8, 9].

King and Garner gave an explanation on the melting point pattern of saturated fatty acids based on crystal diffraction studies and heats of melting [8, 10]. According to this explanation, the terminal methyl and carboxyl groups in crystals of fatty acids actually are further apart than in the liquid state. Thus, when a crystal melts, these terminal groups come closer together, and their melting is exothermic. The crystal is held together by the sidewise attraction of methylene groups in the fatty acid chains, and their melting is endothermic. The methylene group attraction increases with the number of these groups per molecule. As the alcohol chain lengthens from methyl through butyl, the additional methylene groups make the end groups take more and more unfavorable positions in the crystal, making the crystal less stable and lowering their melting points. In esters with alcohol groups greater than butyl, the methylene groups have increasing attraction, and their attraction is greater than the effect of the end groups. So the crystal stability and melting point begin to increase as the alcohol chain grows longer. The same theory could apply to oleate esters.

## Viscosity

The esters from enzymatic synthesis were used for viscosity measurements since they had higher purity than those of the alkali catalyzed reaction. The viscosities of oleate esters with normal alcohol chain lengths of 1–10 and BCFAs, plus branched isopropyl oleate in the

temperature range of 0–70°C are given in Table 2. The melting points of the methyl esters of BCFAs were reported in a previous paper [1]. Methyl esters of 15a and 16i melt at -9 and 16°C, respectively. Although the melting point of these branched derivatives are not particularly low, they were chosen for comparison with the oleate esters. The viscosities of dodecyl oleate and oleyl oleate were not measured because of limited amounts of materials. Viscosity increased with the chain length of the esterified alcohol and with decreasing temperature. The viscosity of the branched isopropyl oleate was close to its straight-chain counterpart, propyl oleate. The branch on the alcohol moieties does not affect viscosity very much. This agrees with Knothes's study [11]. Viscosities of methyl esters of 15a and 16i had viscosities close to those of their straight-chain counterparts as well as that of isopropyl oleate. Such branches on the acid moieties of these esters did not greatly affect viscosity.

The oleate esters and BCFAs methyl esters all behave like Newtonian fluids. At 25°C, the viscosities remain constant while increasing shear rate from 20 to 200 rpm.

Numerous equations have been proposed to fit the change in viscosity with temperature [12]. Many of these equations are based on the idea that energy of activation has to be added to the molecules of a liquid for them to flow past each other. Such equations are analogous to the Arrhenius equation for the rate of a chemical reaction and take the form:

$$\ln \eta = \ln A - \frac{E_a}{RT} \qquad (1)$$

where $\eta$ is the viscosity and $A$ is a constant, $E_a$ is the energy of activation, $T$ is the temperature in degrees Kelvin and $R$ is the gas constant. Ln $\eta$ calculated by versions of this equation gives a straight line for ln $\eta$ versus $1/T$ that poorly fit the experimental data, which gives a very slightly curved line for ln $\eta$ versus $1/T$. We considered that the fit for long linear molecules such as oleate esters might be improved by considering the spatial orientation of the activated molecules as in Erying's rate equation [13]. This equation is often simplified by assuming changes in entropy for the activated state are negligible. Then

$$\ln \eta = \ln\left(\frac{R}{Nh}\right) + \ln T - \frac{E_a}{RT} \qquad (2)$$

where $N$ is Avogadro's number and $h$ is Planck's constant. The fit of the logarithm of the viscosity data was improved by the inclusion of the ln$T$ term, but the fit was still imperfect.

By using our viscosity data and appropriate values of $R$, $N$ and $h$, we were able to calculate $E_a$ at the various temperatures for our esters. Such a treatment revealed that $E_a$ was not a constant but was almost a linear function of $T$. Further study showed that the fit of the data by this linear equation, like that of Eq. 1 was poorer at the extreme temperatures and that the fit could be improved from $R^2 = 0.9999$ to 1 by using a polynomial, such that

$$E_a = B + CT + DT^2 \qquad (3)$$

where B, C and D are constants. Thus,

$$\ln \eta = \ln\left(\frac{R}{Nh}\right) + \ln T - \left[\frac{B + CT + DT^2}{RT}\right] \qquad (4)$$

It may be that for long linear molecules, such as these esters, $E_a$ is a function of $T$ because as temperature increases, the probability of the molecule departing from linearity increases. Departure from linearity entails a corresponding increase in the energy needed to move one molecule past another. This equation's $DT^2$ term also succeeded in fitting the curve of the experimental ln $\eta$ versus $1/T$ data.

The fit of data by Eq. 4 is not strongly dependent on the absolute value of ln[$R$/($Nh$)], as $B$, $C$ and $D$ can change to make the equation fit when various values of this constant are used. We used 28.3651 for ln[$R$/($Nh$)] when $\eta$ was in centipoise.

Plots of $B$, $C$, and $D$ versus $n$, the chain length of the alcohol portion of the esters are generally linear functions but show considerable scatter. This may be the result of varying amounts of impurities in the preparations that we could not detect by the methods we used. Alternatively, this scatter may reflect differences in the favored configuration of the various esters possibly reflecting the interplay of methylene and end groups noted by King and Garner [8, 9] in melting points. However, the errors caused by scatter in the $B$, $C$, and $D$ plots are relatively insignificant. If one fits these plots with the best straight line for the three plots one gets the following equation:

$$E_a = -(833.31n + 33089) + (3.1648n + 419.28)T - (0.0039n + 0.1297)T^2 \qquad (5)$$

where $n$ is the chain length of the alcohol in the oleate esters. Using this equation to obtain $E_a$ and substituting it in Eq. 2 allow one to calculate the viscosity of the esters with less than 1% deviation from the experimental value. The fit is worst for the 0°C values but even these

values differ from the experimental values by an average of 2.2% and do not exceed 4%. The viscometer has an error of ±1% under the conditions used for our measurements.

An example of the fit of decyl oleate data is given in Fig. 2. Thus, these equations allow predicting the viscosity at other temperatures and longer chain lengths. This equation was developed for the oleate esters of straight chain alcohols, but fit equally well for isopropyl ester and the methyl esters of BCFAs. The equations for isopropyl oleate, methyl esters of 16i and 15a, respectively, are:

$$E_a = -38561 + 445.57T - 0.1632T^2 \quad (6)$$

$$E_a = -53717 + 541.21T - 0.3103T^2 \quad (7)$$

$$E_a = -30266 + 408.02T - 0.117T^2 \quad (8)$$

**Acknowledgments**

We acknowledge James Cornette for advice about the viscosity equation, and to the Center for Crops Utilization Research and the Plant Science Institute at Iowa State University for financial support.

**References**

1. Yao L, Hammond EG (2006) Isolation and melting properties of branched fatty esters from Lanolin. J Am Oil Chem Soc 83:547–552

2. Fatemi SH, Hammond EG (1980) Analysis of oleate, linoleate and linoleate hydroperoxides in oxidized ester mixtures. Lipids 15:379–385

3. Downing DT, Kranz ZH, Murray KE (1960) Studies in waxes. XIV. An investigation of the aliphatic constituents of hydrolysed wool wax by gas chromatography. Aust J Chem 13:80–94

4. Lee I, Johnson LA, Hammond EG (1995) Use of branched-chain esters to reduce the crystallization temperature of BioDiesel. J Am Oil Chem Soc 72:1155–1160

5. AOCS (1989) Official Methods and Recommended Practices of the American Oil Chemists Society, 4th edn. American Oil Chemists' Society, Champaign, Method Cj, pp 1–94 (94)

6. Vieville C, Mouloungui Z, Gaset A (1995) Synthesis and analysis of the C1-C18 alkyl oleates. Chem Phys Lipids 75:101–108

7. Candy L, Vaca-Garcia C, Borredon E (2005) Synthesis and characterization of oleic succinic anhydrides: structure–property relations. J Am Oil Chem Soc 82:271–277

8. Bailey AE (1950) Melting and Solidification of Fats. Interscience Publishers, Inc., New York, pp 146–147

9. Gunstone FD (1994) Fatty acid structure. In: Gunstone FD, Harwood JL, Padley FB (eds) The Lipid Handbook. Chapman & Hall, London, pp 1

10. King AM, Garner WE (1936) Melting points of long-chain carbon compounds. J Chem Soc 1936:1368–1372

11. Knothe G, Steidley KR (2005) Kinematic viscosity of biodiesel fuel components and related compounds. Influence of compound structure and comparison to petrodiesel fuel components. Fuel 84:1059–1065

12. Fasina OO, Hallman H, Craig-Schmidt M, Clements C (2006) Predicting temperature-dependence viscosity of vegetable oils from fatty acid composition. J Am Oil Chem Soc 83:899–903

13. Getman FH, Daniels F (1943) Outlines of Physical Chemistry, 7th edn. Wiley, New York, pp 374

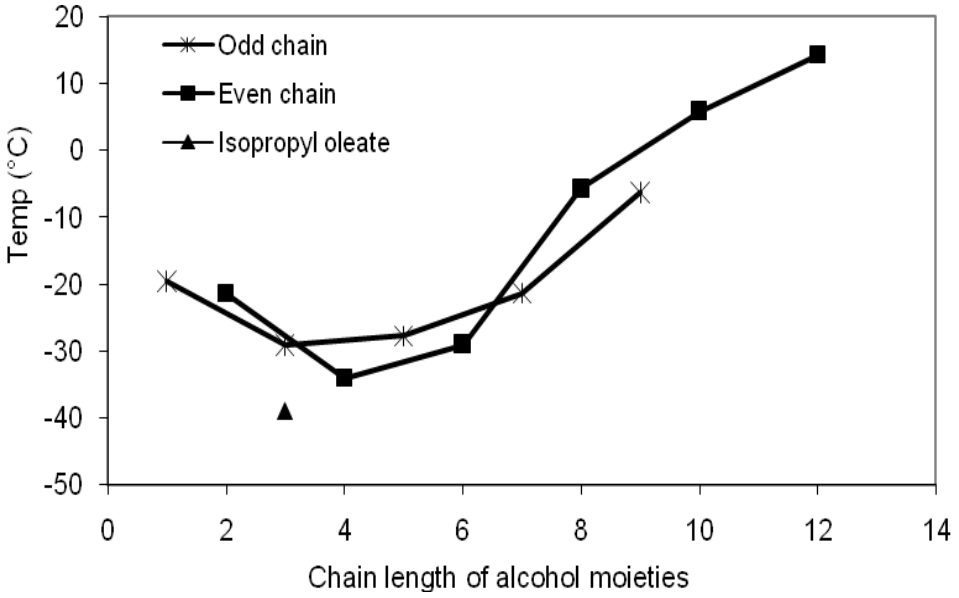



**Fig. 1** Effects of chain length on the melting points ($T_{com}$) of oleate esters

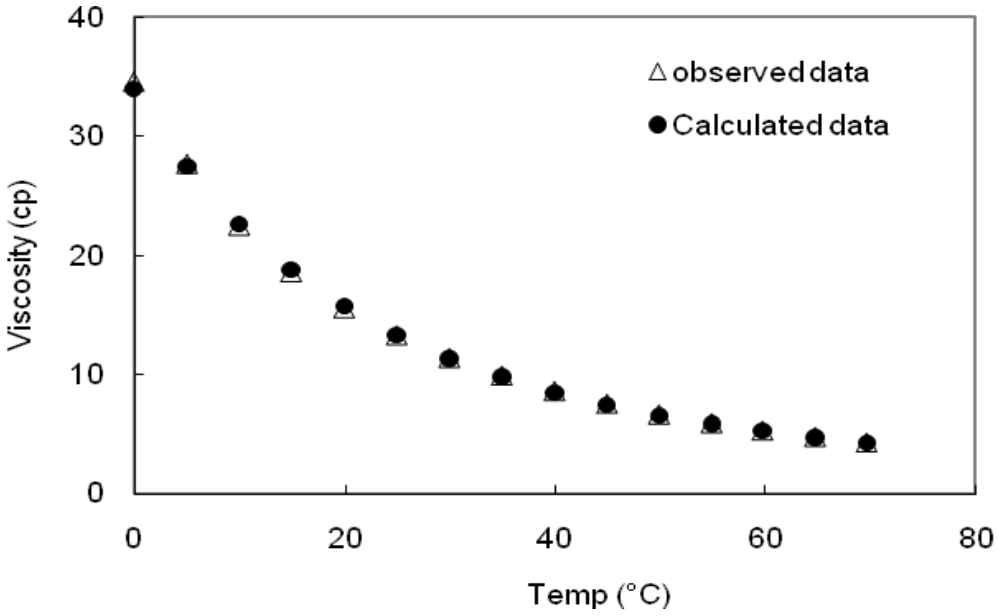



**Fig. 2** Fit of observed viscosity data of decyl oleate at various temperatures with calculated data of Eq. 2 and Eq. 5.

**Table 1** Melting onset ($T_{onset}$), peak ($T_p$) and completion ($T_{com}$) temperatures of oleate esters

| Alcohol moieties | $T_{onset}$ (°C) | $T_p$ (°C) | $T_{com}$ (°C) |
|---|---|---|---|
| Methyl | -20.3 | -17.3 | -16.3 |
| Ethyl | -23.1 | -19.0 | -18.1 |
| Propyl | -32.5 | -28.3 | -27.2 |
| Butyl | -35.7 | -32.8 | -31.7 |
| Pentyl | -29.2 | -26.3 | -25.2 |
| Hexyl | -30.3 | -27.0 | -25.8 |
| Heptyl | -18.3 | -14.7 | -13.1 |
| Octyl | -9.3 | -4.1 | -2.9 |
| Nonyl | -9.4 | -5.6 | -4.4 |
| Decyl | 2.8 | 6.8 | 8.2 |
| Dodecyl | 12.9 | 17.3 | 18.4 |
| Oleyl | -7.1 | -2.9 | -1.5 |
| Isopropyl | -37.7 | -34.2 | -33.4 |

**Table 2** Viscosities of oleate esters, BCFAs methyl ester and saturated straight-chain FAME

| Temp (°C) | Viscosity (cp) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Methyl | Ethyl | Propyl | Butyl | Pentyl | Hexyl | Heptyl | Octyl | Nonyl | Decyl | Isop | 15a | 15n | 16i | 16n |
| 0.00 | 12.30 | 13.60 | 15.70 | 17.20 | 19.20 | 21.80 | 25.00 | 27.45 | 30.60 | 34.60 | 15.40 | 7.97 | - | - | - |
| 5.05 | 10.20 | 11.30 | 13.00 | 14.10 | 15.65 | 17.80 | 20.20 | 22.25 | 24.60 | 27.70 | 12.60 | 6.85 | - | - | - |
| 10.00 | 8.64 | 9.60 | 10.95 | 11.80 | 13.00 | 14.70 | 16.60 | 18.20 | 20.10 | 22.50 | 10.50 | 5.90 | - | 9.86 | - |
| 14.95 | 7.39 | 8.17 | 9.25 | 9.98 | 10.95 | 12.30 | 13.90 | 15.20 | 16.70 | 18.60 | 8.89 | 5.11 | - | 8.33 | - |
| 20.00 | 6.37 | 7.05 | 7.96 | 8.55 | 9.32 | 10.40 | 11.70 | 12.80 | 14.30 | 15.55 | 7.63 | 4.48 | 5.05 | 7.06 | - |
| 25.00 | 5.66 | 6.25 | 6.98 | 7.48 | 8.23 | 9.12 | 10.15 | 11.10 | 12.10 | 13.30 | 6.57 | 4.03 | 4.42 | 5.96 | - |
| 30.00 | 4.97 | 5.48 | 6.08 | 6.52 | 7.15 | 7.88 | 8.77 | 9.53 | 10.40 | 11.40 | 5.67 | 3.61 | 3.91 | 4.94 | 4.48 |
| 35.00 | 4.39 | 4.83 | 5.36 | 5.75 | 6.25 | 6.89 | 7.63 | 8.30 | 9.03 | 9.91 | 4.98 | 3.22 | 3.49 | 4.31 | 3.94 |
| 40.00 | 3.91 | 4.30 | 4.75 | 5.08 | 5.50 | 6.05 | 6.67 | 7.27 | 7.89 | 8.62 | 4.40 | 2.91 | 3.11 | 3.79 | 3.53 |
| 45.00 | 3.50 | 3.84 | 4.24 | 4.54 | 4.88 | 5.36 | 5.88 | 6.39 | 6.95 | 7.54 | 3.89 | 2.65 | 2.8 | 3.36 | 3.14 |
| 49.95 | 3.17 | 3.46 | 3.80 | 4.10 | 4.39 | 4.78 | 5.23 | 5.67 | 6.15 | 6.64 | 3.50 | 2.42 | 2.54 | 3.03 | 286 |
| 54.95 | 2.87 | 3.14 | 3.43 | 3.69 | 3.92 | 4.26 | 4.68 | 5.06 | 5.51 | 5.91 | 3.15 | 2.22 | 2.32 | 2.75 | 2.6 |
| 59.80 | 2.62 | 2.85 | 3.12 | 3.34 | 3.56 | 3.84 | 4.18 | 4.55 | 4.91 | 5.28 | 2.86 | 2.05 | 2.13 | 2.49 | 2.38 |
| 64.80 | 2.40 | 2.62 | 2.84 | 3.04 | 3.22 | 3.49 | 3.79 | 4.11 | 4.44 | 4.76 | 2.59 | 1.89 | 1.96 | 2.29 | 2.19 |
| 69.70 | 2.21 | 2.42 | 2.62 | 2.78 | 2.95 | 3.19 | 3.46 | 3.71 | 4.00 | 4.29 | 2.38 | 1.76 | 1.81 | 2.11 | 2.02 |

Note: 15a – 12-methyltetradecanoic acid methyl ester; 15n – pentadecanoic acid methyl ester; 16i – 14-methylpentadecanoic acid methyl ester; 16n – hexadecanoic acid methyl ester; isop – isopropyl oleate; The viscosities of 15n, 16i, and 16n were measured above their individual melting points.

# CHAPTER 3. SYNTHESIS OF RICINOLEATE ESTERS AS POTENTIAL BIOLUBRICANTS AND THEIR PHYSICAL PROPERTIES

A paper to be submitted to

The *Journal of American Oil Chemists' Society*

Linxing Yao[1,2], Earl G. Hammond[1,3], Tong Wang[1]

**Abstract** The urgent need for replacing petroleum-based lubricants with sustainable, environmental-friendly alternatives has encouraged the developments of vegetable oil-based materials as biolubricants. Our studies have focused primarily on molecules that might be produced by biosynthesis in genetically-altered oilseed plants. Lubricants based on common fatty acids often have high melting points if saturated or poor oxidative stability if unsaturated. These problems can be avoided by using monounsaturated fatty acid. In this study, we synthesized various ricinoleate esters by transesterification and investigated their physical properties. The products that we studied included: (1) ricinoleic acid esterified with normal alcohols of chain lengths 1 to 5 as well as the isopropyl ester, (2) 12-acetylricinoleate esters, (3) methyl ricinoleate esterified on the 12-position with normal carboxyl acids of chain lengths 4 to 10, (4) methyl or isopropyl 12-hydroxystearate esterified on the 12-position with acetic or butyric acid. The purity of all the products was about 98-99% by gas chromatography. Some of these compounds showed melting points and viscosities that would be suitable for uses as biolubricants. Ricinoleate esters melted between -3 and -29°C and had viscosities of ~130 to 180 cp at -5°C, and 4 cp at 80°C. The addition of a branch on the hydroxyl group of ricinoleate prevented crystallization until the branch was longer than 8-carbons. Methyl and isopropyl esters of 12-acetyl and 12-butyrylstearate melted about 50°C lower than the corresponding 12-hydroxystearate.

---

[1]Graduate student, University Professor Emeritus, and Associate Professor, respectively, Department of Food Science and Human Nutrition, Iowa State University.
[2]Primary researcher and author.
[3]Author for correspondence.

**Keywords** 12-acetylricinoleate esters · 12-hydroxystearate · melting point · ricinoleate · viscosity

## Introduction

The use of vegetable oil-based biolubricants to replace those based on petroleum has received more recent attention because of environmental concerns and the high price of crude petroleum. More than 60% of the lubricants used in the United States become pollutants through spillage, evaporation, and total loss lubrication (i.e. lubrication in which the oil is not collected after use for disposal) [1].

Biolubricants that are produced from natural fats and oils generally are biodegradable and nontoxic. Biolubricants also can lessen America's dependence on foreign oil. Certain properties of conventional vegetable oils, however, have limited their use as biolubricants even though they provide derivatives with excellent lubricity and viscosity indices. Fatty acids often have high melting points if saturated or poor oxidative stability if unsaturated, traits that are particularly undesirable in lubricant applications. Various chemical modifications of conventional vegetable oils improve their lubrication performance, such as epoxidation, partial hydrogenation, oligomerization, etc [2]. Alternatively, fatty acids with desired lubrication properties might be produced in plants that have been modified by genetic engineering techniques [3]. Potentially such an approach could produce major savings [4]. The goal of this research was to design and identify molecules that might be biosynthesized in oilseed crops that would possess suitable lubrication properties, i.e. low melting point, suitable viscosity, great stability to oxidation, and good lubricity. Previously, we have reported that certain fatty esters with iso- or anteiso- methyl branches or oleate monoesters might be potential biolubricant targets for genetically engineered oilseeds [5,6]. But these candidates generally have low viscosities that might limit their application in lubrication. In this study, we synthesized and measured properties of several ricinoleate esters that have higher viscosities than those we have studied previously.

## Materials and Methods

### Materials

Castor oil was purchased from a local pharmacy. Potassium isopropylate (19%) solution in isopropanol and potassium propylate (20%) solution in propanol were purchased from BASF Corp. (Evans City, PA). Silica gel (40–140 Mesh) was from J.T.Baker (Phillipsburg, NJ, USA). Other chemicals were purchased from Sigma-Aldrich (St Louis, MO, USA).

### Methods

#### *Purification of Methyl Ricinoleate*

About 20 g of castor oil was dissolved in 300 ml methanol containing 2% of 5 N sodium methoxide. The mixture was vigorously stirred overnight and the reaction was stopped with the addition of acetic acid. The methyl ester was extracted with hexane and washed with 2% aqueous acetic acid and water. The resultant methyl ester contained 86.7% methyl ricinoleate (MeR), 5.3% methyl linolenate, 3.7% methyl oleate, 0.7% methyl palmitate, 0.7% dihydroxystearate, 0.3% methyl eicosanoate and minor impurities by gas chromatography (GC).

MeR was freed of non-hydroxy FAMEs by a countercurrent-current fractionation method adapted from Berdeaux et al [7]. The crude methyl ester (30 g) of castor oil was dissolved in 200 ml hexane and washed with 101 ml of methanol-water-acetic acid, 90/10/1, v/v/v). The methanol phase was then transferred to a second funnel and washed with 200 ml fresh hexane. After being washed with another 200 ml fresh hexane in a third funnel, the methanol phase was collected as fraction 1. The same procedure was repeated 12 times. Then 1 ml of each of the 12 fractions was extracted with hexane (2 ml) and water (5 ml), and the hexane layer was analyzed by GC. Fractions with >98.5% (GC%) of MeR were combined and partitioned between hexane and 2% aqueous acetic acid. After removal of hexane, the pure MeR was stored at -5°C.

Silica gel chromatography (0.1 g oil/1 g silica) also was used to purify a small amount of MeR (< 5 g). Non-hydroxy FAMEs and MeR were eluted separately with the solution of hexane and diethyl ether at ratios of 95:5 (v/v) and 80:20 (v/v), respectively. The purity of resultant MeR was greater than 99% (GC%). Less than 0.5% of methyl dihydroxystearate was present and no further purification was performed.

*Synthesis of Ricinoleate Esters with Alcohol Chain Length 2 to 5 (Compounds 2-6)*

Ethyl (Et), propyl (Pr), butyl (Bu), pentyl (Pe), and isopropyl (iPr) ricinoleate were synthesized via the same procedure as methyl ricinoleate but at a smaller scale (5-8 g) and catalyzed by the corresponding metal alkoxides. The reactions of isopropyl and propyl esters of ricinoleic acid were catalyzed by potassium isopropylate and potassium propylate, respectively. The catalysts for esters with alcohols longer than propyl were generated by reacting sodium metal with various alcohols. The procedures have been described by Yao et al [6]. The resultant esters were purified with silica gel chromatography.

*Synthesis of 12-Hydroxystearic Acid Methyl Ester and its Isopropyl Ester with Novozyme 435 (Compounds 18 and 19)*

To examine the effects of saturated structures on the physical properties of ricinoleate derivatives, several esters derived from 12-hydroxystearic acid were synthesized. Enzymatic methods were explored for the synthesis of 12-hydroxystearic acid methyl ester (MeS) and isopropyl ester (IsS). The reaction took place at room temperature for 1 day with the enzyme load at 5% of the alcohol and the reactants' molar ratio at 1:50 (12-hydroxystearic acid : alcohol)

*Synthesis of 12-Acetylricinoleate Esters of Various Alcohol Chain Lengths (Compounds 7-11)*

MeR (6 mM) was reacted with methyl acetate (300 mM) catalyzed by sodium methoxide (2% by weight of the methyl acetate) for 3 hr at 25°C with stirring. The product was washed

with 2% aqueous acetic acid and water, and extracted with hexane. Methyl acetate was removed by distillation. The product was purified by chromatography on silica gel. The non-polar methyl 12-acetylricinoleate (MeR-Ac) was eluted with 5% diethyl ether in hexane. Other 12-acetylricinoleate esters with longer alcohol chains, compounds 8-11, were synthesized by the same procedure using the corresponding acetate esters, appropriate ricinoleate ester and sodium alkoxide. Ethyl 12-acetylricinoleate (EtR-Ac), for example, was obtained from the reaction of ethyl acetate and ethyl ricinoleate (EtR) and catalyzed by sodium ethoxide.

A small amount (<2% by GC) of ricinoleate estolides dimers was inevitably produced during the synthesis of 12-acetylricinoleate esters even though a large excess of acetates was used and the estolides further interesterified with the acetates to form nonpolar polymers which were difficult to remove from the products.

*Synthesis of 12-Acylated Ricinoleates and Stearates with Short Chain Fatty Acids (Compounds 7, 12-15, 20-23)*

Because of the problems encountered in the previous section, pyridine, a weak base catalyst, was used in the reaction between the 12-hydroxy group on MeR and acetic and butyric anhydride. A molar ratio of 1: 1.2: 2.4 of MeR: acid anhydride: pyridine was used for the synthesis of compounds 7 and 12. Following the Sharma et al procedure [8], a solution of MeR and pyridine in methylene chloride was cooled in ice water, and then the acid anhydride was added dropwise. When no more heat was released, the reaction was left at room temperature for 2 days with stirring. Next, the mixture was diluted with hexane, and washed twice with 0.5M sulfuric acid and saturated aqueous sodium bicarbonate and three times with water. After removal of solvent by rotoevaporation, the acid anhydride was removed under vacuum. The residual mixture of 12-acylated ricinoleate ester and unreacted MeR was separated with silica gel chromatography. The desired product was eluted with 5% diethyl ether in hexane. Similar synthesis strategies were applied for isopropyl 12-butyrylricinoleate (IsR-Bu), compound (14), and the acylation of methyl 12-hydroxystearate with acetic and butyric anhydrides (compounds 20-23).

Methyl 12-hexanoylricinoleate (MeR-Hx) (compound 13), and methyl 12-octanoylricinoleate (MeR-Oc) (compound 15) were synthesized by esterification of MeR with hexanoic acid and octanoic acid, respectively, catalyzed by 4-dimethylaminopyridine (DMAP) and 1,1-dicyclohexylcarbodiimide (DCC), following the procedure of Kodali et al [9]. To a solution of 0.9 g of MeR (3 mM) in methylene chloride (15 ml), hexanoic acid (0.4 g; 3.6 mM) and DMAP (0.4 g; 3 mM) were added with stirring. Then DCC (0.7 g; 3.6 mM) dissolved in methylene chloride (5 ml) was added dropwise. The reaction was carried out at room temperature for 3 hr. After the precipitated dicyclohexylurea was removed by filtration, the ester was extracted with hexane in the same manner as in the pyridine-catalyzed reaction above. Crystallized DMAP in hexane was filtered and the residual hexane was evaporated.

*Synthesis of Methyl 12-Acylated Ricinoleates with Decanoic and 10-Undecenoic Acids (Compounds 16 and 17)*

Methyl 12-decanoylricinoleate (compound 16) was synthesized by reacting methyl ricinoleate with methyl decanoate at a molar ratio of 1:5, and catalyzed by 2% sodium methoxide. The excess of methyl decanoate was distilled off. GC analysis showed that the product contained ~80% methyl 12-decanoylricinoleate (MeR-10,) and trace amounts of MeR (10%), methyl ricinoleate estolides (MeR-Estolide, 2%) and methyl 12-decanoylricinoleate estolides (MeR-Estolide-10, 8%). The polar constituents, MeR and MeR-Estolide were removed by silica gel chromatography. The nonpolar MeR-Estolide-10 was removed by silver ion-silica gel chromatography.

The preparation of silver ion-silica gel chromatograph containing 20-30% silver nitrate was adapted from Ghebreyessus et al [10]. About 140 g silica gel was added to a 300 ml of 22% aqueous solution of silver nitrate. The mixture was well stirred for 10 min and dried at 110°C for ~5 hr. The dry, hot, free-flowing powder was stored in an amber glass bottle. A gradient elution of 45-60% diethyl ether in hexane washed out MeR-10 that had one double bond, and left MeR-Estolide-10 that had two double bonds in the column.

The synthesis and purification of methyl 12-undec-10-enoylricnoleate (compound 17, MeR-11u) were similar to those for compound 16. A gradient elution of 85-90% ether/hexane collected MeR-11u from the silver ion-silica gel column.

*Instrument Analysis*

GC analysis was done on a HP 5890 Series II instrument (Hewlett-Packer, PA, USA) with a SPB-1 fused silica column (15 m x 0.25 mm x 0.25 μm) (Supelco, Bellefonte, PA, USA). The injector and flame detector were at 300°C, and the oven temperature was programmed from 100 to 300°C at a rate of 10°C/min. Temperature was then held at 300°C for 10 min.

The structures of the ricinoleate esters in $CDCl_3$ were confirmed by $^1H$ NMR with a Varian VXR-400 NMR spectrometer (Varian Inc., Palo Alto, CA, USA).

Melting points of the synthesized esters were measured with a DSC7 differential scanning calorimeter equipped with an Intracooler System I (Perkin Elmer, Norwalk, CT, USA). The temperature programs and calibrations with indium and n-decane were described previously [6]. Visual observations of samples cooled with dry ice-acetone were used to determine the approximate gelation temperature for those did not crystallize in DSC.

The viscosities of the various ricinoleate esters in the temperature range 0 to 80°C were determined with a Brookfield DV II + viscometer (Brookfield Inc., Stoughton, MA, USA) using a CP42 cone spindle. The instrument condition, temperature calibration, and procedure for measurement were described previously [6].

The viscosities of 12-acetylricinoleate esters at temperature range of -34 to 0°C were determined with the same viscometer and spindle. The temperature was controlled with a Brinkmann RK 20 refrigerated circulator (Westbury, NY, USA) and was monitored with a total immersion Fisherbrand ASTM thermometer (Fisher Scientific, Pittsburgh, PA, USA) with the temperature of -38 to 2°C, which was inserted in a tube on the return line of the circulating water bath. Data was collected between -34 and 0°C at 5°C intervals. Viscosities were recorded after equilibrating at the desired temperature for 5 min and at the maximum possible torque.

**Results and discussion**

Purification of Methyl Ricinoleate

The purities and yields of 12 fractions collected from the methanol phases in the countercurrent distribution are shown in Fig. 1. The purities of fraction 3 to 11 were ≥ 98% (GC %), and these fractions contained most of the methyl ricinoleate. Fractions 1, 11 and 12 were discarded and the rest were combined for use in other syntheses. This purification of MeR by the countercurrent fractionation can be quite economical if the methanol and hexane are recovered and recycled.

Synthesis

Various interesterification methods were explored. Both sodium alkoxides and Novozyme 435 were able to catalyze the production of ricinoleate and 12-hydroxystearate monoesters with alcohols in good yield. Sodium alkoxide more efficiently catalyzed the reaction with triacylglycerides, while Novozyme 435 worked better in esterifying these FFA and alcohols. Both methods produced ricinoleate estolides, especially if reaction time was prolonged.

The strategy used to synthesize 12-acetylricinoleates esterified with various alcohols was to interesterify the desired ester of ricinoleate with the acetate ester of the same alcohol. A large molar excess of the alcohol acetate was used to suppress estolide formation which acetylated the 12-position. The reaction could generally be finished quickly at low cost. The yields of 12-acetylricinoleic esters were 85-87% (Fig. 2). The disadvantage of using sodium methoxide to catalyze such reactions was that 1~2% of nonpolar estolides remained after purification by silica chromatography.

To examine whether estolide esters present in our samples significantly affected the physical properties of the acylated ricinoleate esters, pyridine also was used as catalyst to synthesize MeR-Ac for melting point measurements. The products made in this way did not contain estolides.

The use of DMAP and DCC to catalyze the reaction between hexanoic acid (and other FFAs) and MeR could save the steps converting FFAs to their anhydrides or chlorides. The yields of the reactions catalyzed by pyridine or DMAP are shown in Fig. 2. In general, the reactions catalyzed by DMAP gave higher yield than those catalyzed by pyridine, compounds 13 and 15 had yields above 95%. Short chain compounds had higher yields than long chain compounds if the same catalyst was used, i.e. MeR-Ac had higher yields than MeR-Bu when both were catalyzed by pyridine. Reactions between MeR and a long chain fatty ester, such as methyl decanoate or methyl 10-undecylenate, were fast and had high yields (>80%) if catalyzed by sodium methoxide, but brought troubles in purification when high purity was important. Silver ion-silica gel chromatography was not very efficient in separating of compounds with double bonds far from each other, such as compounds 16 and 17.

Effects of Chemical Structures on Melting Points

The melting points (completion of melting) of ricinoleate and 12-hydroxystearate esters are shown in Table 1. Butyl ricinoleate melted the lowest at -29.4°C. The melting points of straight-chain ricinoleates decreased with chain length until butyl ester and then the melting point increased with chain length. This V-shaped melting point pattern has been found for oleate esters, stearate esters, and saturated free fatty acids [6,11]. References [5,11,12] should be consulted for speculation about the cause of this phenomena. The melting point of isopropyl ricinoleate, which melts at -20.3°C, is very similar to that of the corresponding straight chain, propyl ricinoleate, which melts at -19.4°C. For several types of fatty acid esters, isopropyl esters melted significantly lower than the propyl ester. Presumably the isopropyl group interferes with the close packing of alkyl chains. Isopropyl and propyl ricinoleate's similar melting points suggest that the hydroxy group of the ricinoleate dominates its crystal packing pattern and makes the effect of the isopropyl branch negligible.

Figure.3 shows that the melting points of the ricinoleate esters with n-alcohols of various chain lengths are slightly higher than that of the corresponding oleate esters. Similarly, the reported melting point for 12-hydroxystearic acid [13] is slightly higher than

that of stearic acid. These observations suggest that a hydroxyl group slightly increases the melting point of a fatty acid or its esters.

The ricinoleate esters whose 12-hydroxyl groups were esterified with acetate, butyrate, hexanoate and octanoate (compound 7-15), did not crystallize in the temperature range we measured (-60 to 0°C). Instead, they formed gel-like solids at -50 to -55°C. Considering that there were 1~2% of estolide esters presented in some of these 12-acetylricinoleates, we compared their melting points with the same compounds that were alternatively synthesized with pyridine and acid anhydride. No crystallization difference was detected between the pure samples and those contaminated with 1-2% estolides. The gelation temperatures observed suggested that the pour points of these ricinoleate derivatives would be below -45°. The measurement of low temperature viscosity discussed in next section also provides evidence that these compounds could flow freely above their gelation temperature. These compounds might be useful as pour point depressants.

To increase the acyl chain length on the 12-position of MeR, MeR was esterified with decanoyl and 10-undecenoyl groups. In contrast to the shorter 12-acyl branches, the decanoyl and 10-undecenoyl derivaties were able to crystallize at low temperature. Seemingly an acyl group that is a little longer than eight or nine carbons allows crystallization. The melting points of MeR-10 and MeR-11u were -14.0 and -18.7°C, respectively. Compared with MeR, which processes a free hydroxy group, the melting points were lowered considerably by adding these longer branches on the 12-position of MeR. The double bond on the 10-undecylenate moieties further depressed the melting point. Possibly the isopropyl esters of these compounds would have even lower melting points than their methyl ester counterparts.

The low temperature properties of 12-hydroxystearate esters also were studied. 12-Hydroxystearic acid can be obtained from hydrogenation of ricinoleic acid. The melting point of this completely saturated compound, which has an 18-carbon chain, can be lowered significantly by acylating the 12-position of hydroxylstearate esters with short-chain fatty acids. As shown in Table 1, methyl 12-hydroxystearate and isopropyl 12-hydroxystearate melt at 59 and 49°C, respectively. The isopropyl group lowered the melting point of the methyl ester by 10°C. Esterifying the 12-hydroxy group with acetate or butyrate lowered the

melting points by ~ 50°C for both the methyl and isopropyl esters. For example, the melting point of isopropyl 12-butyrylstearate is -15.6°C. Similar structures, iso-stearic acid methyl ester and its isopropyl ester, melt at 27.1 and 7.5°C, respectively [5]. Thus, a branch located in the middle of a long fatty acid chain can lower the melting point more effectively than a branch located at the end of the carbon chain.

During DSC measurements, most of the compounds showed polymorphism. Changing the temperature program and letting the compounds temper for a while at the temperature where the highest melting crystal begins to melt, reduced or deleted the peaks of the low temperature polymorphs. So all the melting points reported here are from the highest temperature polymorphic form, i.e. the most stable form. The melting temperatures of other polymorphic forms (if any) of each compound also were included in Table 1.

Effect of Chemical Structures on Viscosities

The viscosities between -5 and 80°C of the products synthesized in this study are shown in Tables 2 and 3. In general, the viscosities of the compounds we have synthesized all increased with carbon numbers and decrease with elevated temperatures. Branched compounds had higher viscosities than their straight-chain counterparts, Viscosities of these compounds at temperature 20, 40 and 80°C are compared and summarized in Fig. 4. At 20 and 40°C, ricinoleate esters with a free hydroxy group had the highest viscosity, 12-acylated ricinoleate esters of medium chain lengths fatty acids were second, and 12-acetylricinoleates have the lowest viscosity. It indicated that the free hydroxy group in the middle of carbon chain significant increased the viscosity. The hydroxy group increased the viscosity of MeR more than the addition of a 10 or 11-carbon chain on the 12 position. At 80°C, the difference in viscosity among these three types of compounds became relatively small.

The viscosities of some 12-acetylricinoleate esters also were measured in the range of -10 to -34° C, and the results are shown in Table 4. Their viscosities at -34°C are not particularly high, which indicated that they still can flow freely at such temperature. Lower temperature (≤ -40°C) measurements were not performed due to the limitations of the equipment. As expected, the longer the carbon chain of the ester on the 1-position, the greater

the viscosity of the ester. Viscosity progressively increased with decreasing temperature and no abrupt increase in viscosities was observed for any compounds in the measurement temperature range.

The viscosity of isopropyl esters showed some interesting changes with temperature relative to other ricinoleate derivatives. In our previous work on branched esters [6] we found that the isopropyl ester behaved similarly to n-propyl ester in viscosity. In the esters of ricinoleic acid shown in Table 2, the isopropyl ester had higher viscosity than the n-propyl esters as well as the butyl ester at 5°C. The isopropyl ester continued to have slightly higher viscosity than the n-propyl ester thoughout the temperature range studied, but the difference in the viscosity of the isopropyl and butyl ester became smaller as the temperature increased, and at 25°C and higher, the butyl esters had a greater viscosity. Table 3 shows a similar relation among the propyl, butyl and isopropyl esters of the 12-acetylricinoleates. The isopropyl ester had a greater viscosity at -5°C but was surpassed by the butyl ester at 20°C. This observation is further complicated by data in Table 4, which shows that at -10°C, the viscosity of isopropyl 12-acetylricinoleate was well below, not only that of the corresponding butyl ester, but also that of the methyl ester. Seemingly, there was a complex interplay on viscosity between the branched isopropyl group and the branch on the 12 position of ricinoleate.

NMR Characterization of the Compounds

The $^{1}$H NMR spectra for ricinoleate esters (Compounds 1-6) varied with the chain length of alcohol moieties. An example of MeR was given as follows. **MeR,** $^{1}$H NMR: δ 5.586-5.466 (2H, m, C9 –C**H**=C**H**−), 3.713 (3H, t, COOC**H**$_3$), 3.659 (1H, m, C12 **H**C−OH), 2.350 (2H, t, C2 −C**H**$_2$), 2.257 (2H, t, C11 −C**H**$_2$), 2.087 (2H, m, C8−C**H**$_2$), 1.664 (2H, m, C3 −C**H**$_2$), 1.511 (2H, m, C13 −C**H**$_2$), ~1.350 (16H, m, C4-7 and C14-17), and 0.932 (3H, t, C18−C**H**$_3$). Other ricinoleate esters differed in the chemical shifts of hydrogens on the carbon adjacent to the ester bond on alcohol portion, as EtR had δ 4.161 (2H, m, COOC**H**$_2$CH$_3$); PrR had δ 4.070 (2H, m, COOC**H**$_2$CH$_2$CH$_3$) and 1.683 (4H, m, C3 −C**H**$_2$ and COOCH$_2$C**H**$_2$CH$_3$); BuR had δ 4.132 (2H, m, COOC**H**$_2$CH$_2$CH$_2$CH$_3$), 1.672 (4H, m, C3 −C**H**$_2$,COOCH$_2$C**H**$_2$CH$_2$CH$_3$)

and 1.367 (18H, m, C4-7, C14-17, and $COOCH_2CH_2C\mathbf{H}_2CH_3$); IsR had δ 5.007 (1H, m, $COOC\mathbf{H}(CH_3)_2$) and 1.306 (22H, m, C4-7, C14-17, and $COOCH(C\mathbf{H}_3)_2$). Acetylated ricinoleate esters had similar $^1$H NMR spectra to the corresponding ricinoleate esters that had same alcohol moieties, except the chemical shift at C12, which was 4.955 (1H, m, C12 $\mathbf{H}C{-}OOCCH_3$). RO-10 had similar $^1$H NMR spectra to MeR-Ac except hydrogen atoms of two hydrocarbon chains were integrated instead of only one hydrocarbon chains. MeR-11u had similar $^1$H NMR spectra to MeR-10 with an extra chemical shift for the two hydrogen atoms on the carbons next to the double bond that located at methyl end of 10-undecylenic acid.

**Acknowledgement**

We thank Plant Science Institute and Center for Crop Utilization Research at Iowa State University for funding.

**References**

1. Erhan SZ, Sharma BK, Perez JM (2006) Oxidation and low temperature stability of vegetable oil-based lubricants. Ind Crops Prod 24: 292-299

2. Wagner H, Luther R, Mang T (2001) Lubricant base fluids based on renewable raw materials- Their catalytic manufacture and modification. Appl Catal A General 221: 429–442

3. Töpfer R, Martini N, Schell J (1995) Modification of plant lipid synthesis. Science 268: 681-686

4. Dowling A, Zakharenko O (2005) Assessing biodiesel's cold flow limitation and gains from re-engineered soybeans. Unpublished data

5. Yao L, Hammond EG (2006) Isolation and melting properties of branched fatty esters from Lanolin. J Am Oil Chem Soc 83:547–552

6. Yao L, Hammond EG, Wang T (2008) Melting points and viscosities of fatty acid esters that are potential targets for engineered oilseed. J Am Oil Chem Soc 85: 77-82

7. Berdeaux O, Christie WW, Gunstone FD, Sebedio JL (1997) Large-scale synthesis of

methyl *cis*-9, *trans*-11-octadecadienoate from methyl ricinoleate. J Am Oil Chem Soc 74: 1011-1015

8. Sharma BK, Adhvaryu A, Liu Z, Erhan SZ (2006) Chemical modification of vegetable oils for lubricant applications. J Am Oil Chem Soc 83:129–136

9. Kodali DR, Atkinson D, Redgrave TG, Small DM (1987) Structure and polymorphism of 18-carbon fatty acyl triacylglycerols: effect of unsaturation and substitution in the 2-position. J Lipid Res 28: 403-413

10. Ghebreyessus KY, Schiltz H, Angelici RJ (2006) Partial separation of polyunsaturated fatty acid esters from FAMEs mixtures by adsorption on silver nitrate-impregnated silica gel. J Am Oil Chem Soc 83: 645-652

11. Bailey AE (1950) Melting and Solidification of Fats. Interscience Publishers, Inc., New York, pp 146–147

12. King AM, Garner WE (1936) Melting points of long-chain carbon compounds. J Chem Soc 1936:1368–1372

13. Zakharkin LI, Guseva VV, Churilova IM (1983) Synthesis of 12-hydroxycarboxylic acids from 11-cyanoundecanoic acid. Zhurnal Organicheskoi Khimii 19: 1866-1869

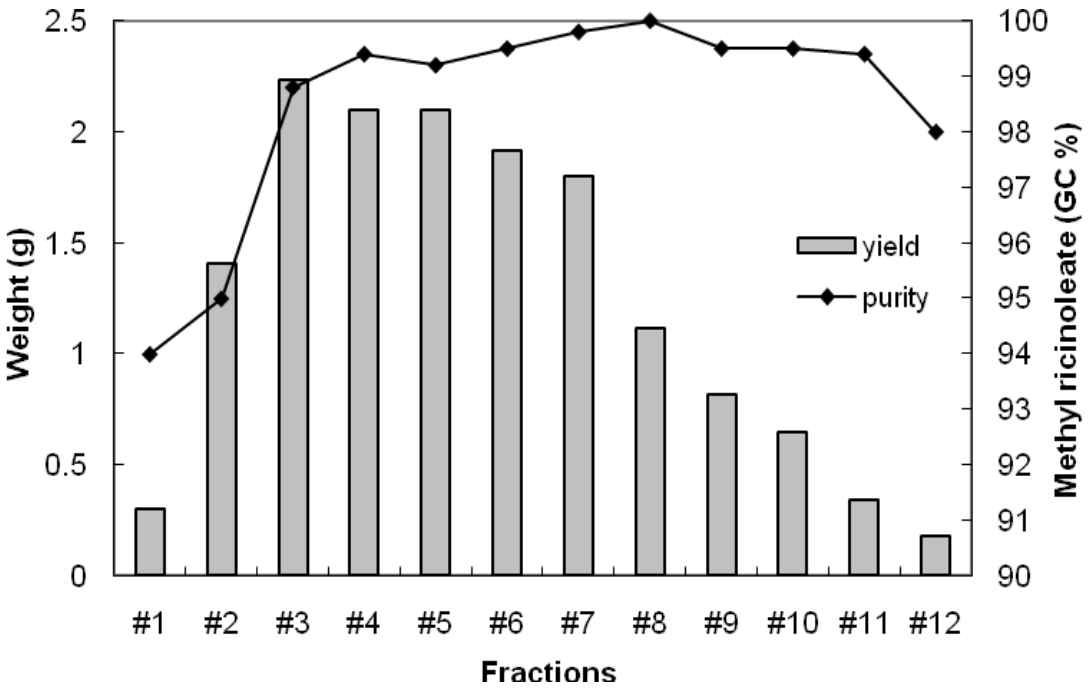
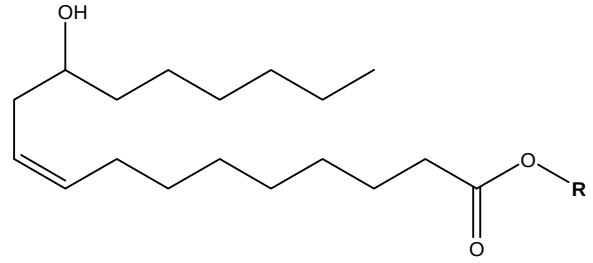


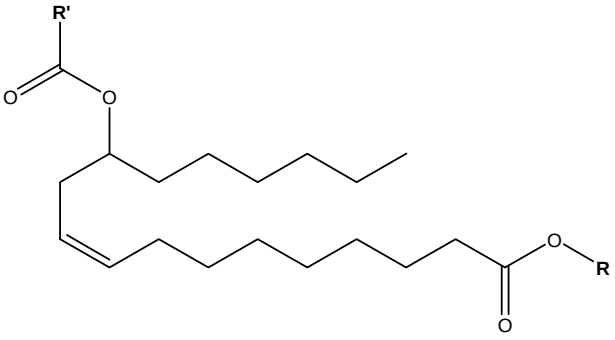

**Fig. 1** The weight and composition of various fractions obtained during the purification of methyl ricinoleate from castor oil methyl esters by countercurrent fractionation using hexane and methanol




**1 - 6**

| | R |
|---|---|
| **1**, | Me |
| **2**, | Et |
| **3**, | Pr |
| **4**, | Bu |
| **5**, | Pe |
| **6**, | *i*Pr |

1-6, yield ~ 99% (c)

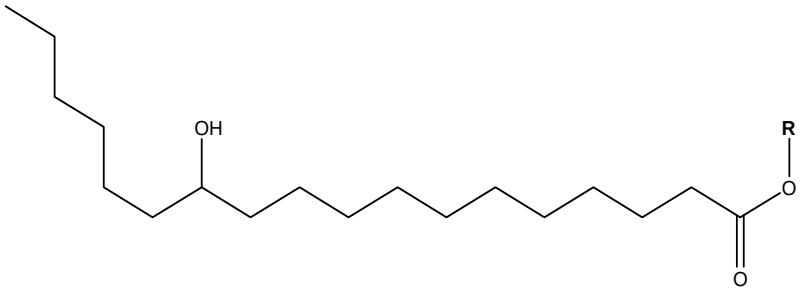



**7 - 17**

| | R | R'COO |
|---|---|---|
| **7**, | Me | Ac |
| **8**, | Et | Ac |
| **9**, | Pr | Ac |
| **10**, | Bu | Ac |
| **11**, | *i*Pr | Ac |

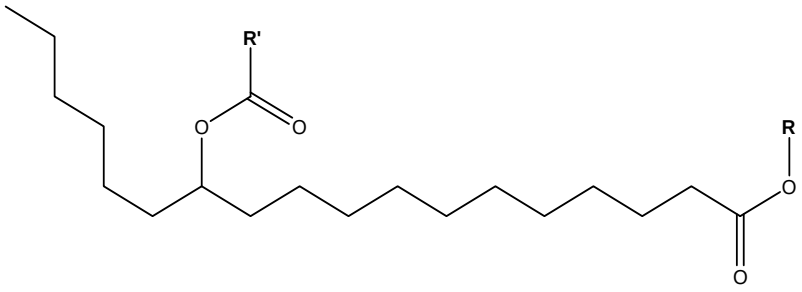
7, 95% (a); 7-11, 85-87%(c)

| | | |
|---|---|---|
| **12**, | Me | Bu, 34% (a) |
| **13**, | Me | Hx, 95% (b) |
| **14**, | *i*Pr | Bu, 25% (a) |
| **15**, | Me | Oc, 95% (b) |
| **16**, | Me | De, 81% (c), 86% (b) |
| **17**, | Me | undec-10-enoate, 80% (c) |




**18 - 19**

| | R |
|---|---|
| **18**, | Me, 95% (d) |
| **19**, | *i*Pr, 95% (d) |




**20 - 23**

| | R | R'COO |
|---|---|---|
| **20**, | Me | Ac, 45% (a) |
| **21**, | Me | Bu, 7% (a) |
| **22**, | *i*Pr | Ac, 71% (a) |
| **23**, | *i*Pr | Bu, 24% (a) |

**Fig. 2** Structures of synthesized ricinoleate and 12-hydroxystearte esters. Reaction catalyzed by (a) pyridine, (b) DMAP and DCC, (c) sodium methoxide (d) Novozyme 435

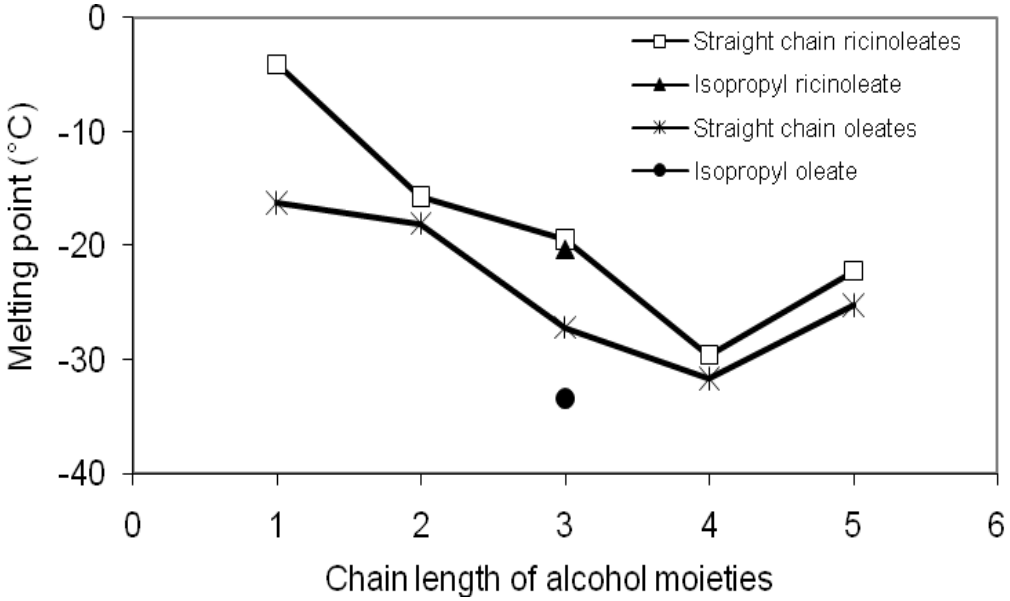



**Fig. 3** Melting points of ricinoleate and oleate esters. The melting points of oleate esters are from Ref. 6

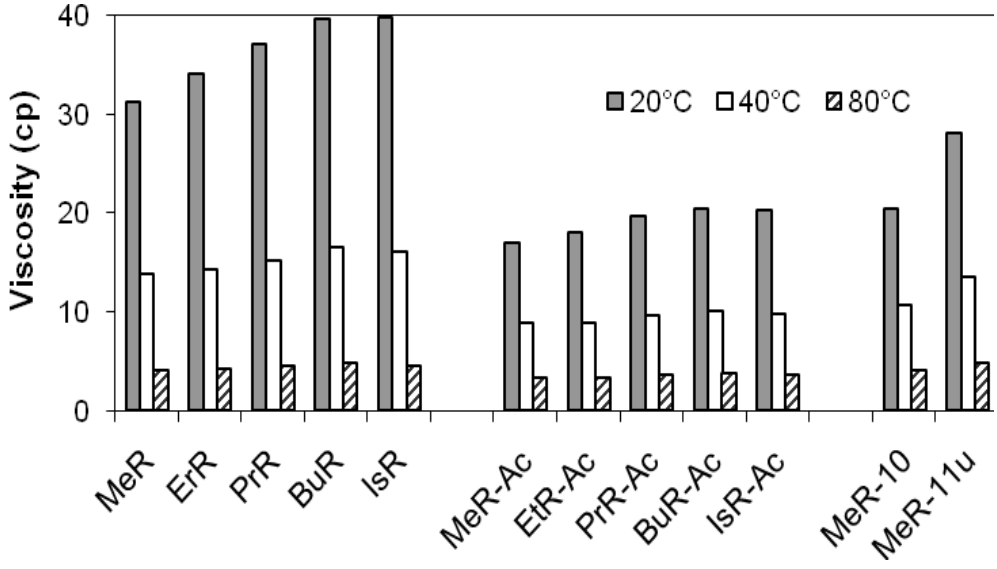



**Fig. 4** Viscosity of various ricinoleate derivatives measured at 20, 40 and 80°C

**Table 1** Melting point of various ricinoleate derivatives

| Compounds number | Chemical name | Polymorph of lower melting point** | $T_{com}$ (°C)*** |
|---|---|---|---|
| 1 | Methyl ricinoleate (MeR) | - | -3.5 |
| 2 | Ethyl ricinoleate (EtR) | - | -15.7 |
| 3 | Propyl ricinoleate (PrR) | - | -19.4 |
| 4 | Butyl ricinoleate (BuR) | -35.5 | -29.4 |
| 5 | Pentyl ricinoleate (PeR) | - | -22.2 |
| 6 | Isopropyl ricinoleate (IsR) | -50.6, -42.6 | -20.3 |
| 16 | Methyl 12-decanoylricinoleate (MeR-10) | -45.0, -33.5 | -14.0 |
| 17 | Methyl 12-undec-10-enylricnoleate (MeR-11u) | -23.8 | -18.7 |
| 18 | Methyl 12-hydroxystearate | 35.3, 52.6 | 59.4 |
| 19 | Isopropyl 12-hydroxystearate | 43.8 | 49.0 |
| 20 | Methyl 12-acetylstearate (MeS-Ac) | -24.0 | -2.5 |
| 21 | Methyl 12-butyrylstearate (MeS-Bu) | -14.1, -3.7 | 3.9 |
| 22 | Isopropyl 12-acetylstearte (IsS-Ac) | -31.2 | -9.8 |
| 23 | Isopropyl 12-butyrylstearate (IsS-Bu) | - | -15.6 |
| 24 | Methyl decanoate* | - | -14.0 |
| 25 | Methyl 10-undecylenate* | - | -18.7 |

*Compound 24 was a commercial sample and compound 25 was prepared by esterifying a commercial sample;
**Melting points of polymorphic peak were taken from the peak temperature on DSC curve;
***$T_{com}$ is the temperature at completion of melting.

**Table 2** Viscosities of ricinoleate esters

| Temp (°C) | Viscosity (cp) | | | | |
|---|---|---|---|---|---|
| | MeR | EtR | PrR | BuR | IsR |
| -5 | 130.8 | 145.6 | 162.3 | 171.6 | 182.6 |
| 0 | 95.1 | 105.1 | 117.1 | 124.1 | 130.2 |
| 5 | 70.2 | 77.8 | 84.9 | 90.8 | 94.2 |
| 10 | 52.7 | 58.1 | 63.1 | 67.6 | 69.1 |
| 15 | 40.0 | 43.8 | 47.9 | 51.3 | 51.8 |
| 20 | 31.2 | 34.0 | 37.0 | 39.6 | 39.8 |
| 25 | 25.9 | 27.0 | 29.0 | 31.4 | 31.3 |
| 30 | 20.0 | 21.5 | 23.1 | 24.9 | 24.7 |
| 35 | 16.8 | 17.3 | 18.6 | 20.1 | 19.7 |
| 40 | 13.8 | 14.2 | 15.2 | 16.5 | 16.1 |
| 45 | 11.5 | 11.8 | 12.8 | 13.6 | 13.2 |
| 50 | 9.70 | 9.87 | 10.8 | 11.4 | 11.0 |
| 55 | 8.25 | 8.40 | 9.10 | 9.71 | 9.28 |
| 60 | 7.05 | 7.15 | 7.76 | 8.30 | 7.92 |
| 65 | 6.09 | 6.19 | 6.70 | 7.13 | 6.81 |
| 70 | 5.30 | 5.39 | 5.81 | 6.21 | 5.89 |
| 75 | 4.67 | 4.72 | 5.11 | 5.43 | 5.15 |
| 80 | 4.11 | 4.19 | 4.51 | 4.80 | 4.55 |

**Table 3** Viscosities of 12-acetylricinoleate esters and methyl ricinoleate diesters

| Temp (°C) | Viscosity (cp) | | | | | | |
|---|---|---|---|---|---|---|---|
| | MeR-Ac | EtR-Ac | PrR-Ac | BuR-Ac | IsR-Ac | MeR-10 | MeR-11u |
| -5 | 55.0 | 59.2 | 66.4 | 68.9 | 73.7 | 65.4 | 96.8 |
| 0 | 40.9 | 45.4 | 50.0 | 52.4 | 54.8 | 50.4 | 76.7 |
| 5 | 31.5 | 35.1 | 38.3 | 40.3 | 41.6 | 39.5 | 56.6 |
| 10 | 24.7 | 27.4 | 29.9 | 31.4 | 32.0 | 31.0 | 45.3 |
| 15 | 20.2 | 22.1 | 23.9 | 25.1 | 25.2 | 25.4 | 35.9 |
| 20 | 16.9 | 18.1 | 19.6 | 20.3 | 20.2 | 20.4 | 28.0 |
| 25 | 14.0 | 14.8 | 16.1 | 16.8 | 16.7 | 17.3 | 22.8 |
| 30 | 12.0 | 12.3 | 13.3 | 14.0 | 13.8 | 14.5 | 19.0 |
| 35 | 10.1 | 10.4 | 11.2 | 11.8 | 11.6 | 12.4 | 15.9 |
| 40 | 8.84 | 8.89 | 9.54 | 10.0 | 9.79 | 10.7 | 13.5 |
| 45 | 7.57 | 7.65 | 8.20 | 8.64 | 8.38 | 9.27 | 11.6 |
| 50 | 6.59 | 6.65 | 7.12 | 7.48 | 7.23 | 8.06 | 9.97 |
| 55 | 5.75 | 5.85 | 6.25 | 6.55 | 6.31 | 7.10 | 8.71 |
| 60 | 5.06 | 5.15 | 5.48 | 5.77 | 5.54 | 6.27 | 7.63 |
| 65 | 4.50 | 4.58 | 4.86 | 5.12 | 4.91 | 5.59 | 6.74 |
| 70 | 4.01 | 4.09 | 4.35 | 4.57 | 4.38 | 5.03 | 5.98 |
| 75 | 3.61 | 3.68 | 3.90 | 4.11 | 3.91 | 4.54 | 5.34 |
| 80 | 3.27 | 3.33 | 3.54 | 3.71 | 3.54 | 4.11 | 4.79 |

**Table 4** Viscosities of 12-acetylricinoleates at low temperature

| Temp (°C) | Viscosity (cp) | | |
|---|---|---|---|
| | MeR-Ac | BuR-Ac | IsR-Ac |
| -10.0 | 88.0 | 91.5 | 81.1 |
| -15.2 | 124.0 | 126.1 | 112.3 |
| -20.4 | 177.8 | 186.0 | 165.0 |
| -24.6 | 257.6 | 263.9 | 234.2 |
| -30.8 | 398.9 | 413.5 | 355.5 |
| -34.9 | 620.9 | 635.9 | 556.1 |

# CHAPTER 4. MELTING POINTS AND VISCOSITIES OF VARIOUS DIOL AND POLYOL ESTERS AS POTENTIAL BIOLUBRICANTS

A paper to be submitted to
*The Journal of American Oil Chemists' Society*

Linxing Yao[1,2], Earl G. Hammond[1,3], Tong Wang[1]

**Abstract** The urgent need for replacing petroleum-based lubricants with alternatives that are sustainable and environmentally friendly has encouraged the development of biolubricants based on vegetable oils. Our studies have focused primarily on molecules that might be produced by biosynthesis in genetically-altered oilseed plants. These molecules also should have proper physical properties, i.e. low melting point and appropriate viscosity. In this study we synthesized and investigated the physical properties of various diol and polyol esters. Some of these esters are not likely to be synthesized via biological mechanisms but they can be made from fatty acids with minimal chemical modifications. The products that we studied included oleate esters of ethylene glycol, 1,2-propanediol, 2,3-butanediol, and pentaerythritol. The 12-methyltetradecanoate and decanoate esters of selected polyols were also made. All the products were >98% (base on GC and TLC). Polyol esters that had a free hydroxyl group had lower melting points than the corresponding completely esterified polyols. The completely esterified polyol esters exhibited less change in viscosity with temperature than those having a free hydroxy group. Some of these compounds had melting points and viscosities that would be suitable for biolubricants, especially 2,3-butanediol monooleate, which melted at -48.6°C and had viscosities of 19.7 cp at 40°C, and 5.4 cp at 80°C.

---

[1]Graduate student, University Professor Emeritus, and Associate Professor, respectively, Department of Food Science and Human Nutrition, Iowa State University.
[2]Primary researcher and author.
[3]Author for correspondence.

**Keywords** 2,3-butanediol · ethylene glycol · melting point · oleic acid · pentaerythritol · 1,2-propanediol · viscosity

## Introduction

Environmental concerns and petroleum shortages have encouraged extensive research on biolubricants in recent years. Biolubricants can be derived from vegetable oils and animal fats, and therefore, their production may be sustainable. They are generally more biodegradable than petroleum-based products, and their use causes less net accumulation of carbon dioxide in the atmosphere.

The use of vegetable oil as lubricants can be traced back several centuries [1], but their inherent shortcomings have prevented significant use as lubricants in modern times. Soybean oil, for example, contains about 50% of linoleate esters, which have two double bonds in their carbon chain. The presence of these double bonds, on the one hand, makes linoleic acid fluid, and prevents it from solidifying above refrigeration temperatures. On the other hand, the double bonds make the fatty acid prone to oxidation and degradation. Both oxidative stability and low-temperature fluidity are important for the oils used in lubrication applications. Among the more than 200 known types of fatty acids, some have structures that might be suitable for lubricants, but few of the traditional vegetable or animal fats and oils make good lubricants.

The development of genetic engineering techniques in recent years has provided the means to alter traditional vegetable oils in interesting ways; the amounts of specific fatty acids can be increased or decreased. It may be possible that a fatty acid with desired lubricant properties can be introduced into another oilseed plants by isolating the genes that are responsible for its synthesis and obtaining strong expression of the exotic fatty acid. Likewise it may be possible to alter the structure in other ways. For example, it might be possible to make methyl or ethyl esters instead of glycerol esters the primary product of oil synthesis. At present the limits of genetic engineering are unknown.

It is our goal to find proper fatty acid and ester candidates for engineered oilseed crops that have suitable properties for lubrication application and could conceivably be

biosynthesized in oilseed plants. In this paper we also study the possible esterification of stable fatty acids with various polyols, some of which might be biosynthesized and others which probably cannot be.

Diesters of fatty acids have been used as synthetic lubricants for years. The commercially available diesters are mostly synthesized by reacting a linear diacid with a branched monofunctional alcohol. The linear hydrocarbon chains of the diester contribute to its viscosity, while the branched ends give a good pour point. One of the disadvantages of such synthetic diesters has been their low molecular weights and correspondingly low viscosities [2]. Their extensive processing required to making and purifying these materials results in high prices.

Diesters made from diols and fatty acids have linear structures similar to those made from diacids, and one would expect them to possess similar properties. Glycol esters have been used as sperm oil substitutes and as centrifugal refrigerant compressor lubricants [3]. Basu et al [3] evaluated the lubrication performance of glycol mono- and diesters of partially hydrogenated soybean oil and found that these glycol esters were suitable as antifriction additives. Monoesters of diols were excellent surfactants due to the free hydroxyl groups present. Such surfactant properties would be expected to improve the oil's lubricity.

Although diesters provide excellent lubricating effects, their thermal stabilities are surpassed by the polyol esters such as pentaerythritol, which has no hydrogen atom on the β carbon. This feature provides higher degrees of thermal stability than glycerol-based lubricants. Moreover, the branched configuration of pentaerythritol prevents alignment of carbon chains during crystallization, which lowers the melting point of the corresponding ester. Pentaerythritol esters of fatty acids with medium chain lengths ($C_5$-$C_9$) are currently used in gas turbine engines [4].

In this paper we report the synthesis of both partial and fully esterified diols and pentaerythritol and measured their physical properties important for lubricant applications.

## Materials and methods

### Materials

Silica gel (40–140 Mesh) was from J.T. Baker (Phillipsburg, NJ, USA). Silica gel G Uniplates (250 lm) with inorganic binder for thin layer chromatography (TLC) were purchased from Alltech (State College, PA, USA). Other chemicals including Amberlite A21 ion-exchange resin were purchased from Sigma-Aldrich (St Louis, MO, USA).

## Methods

### *Enzymatic Syntheses of Diol Esters*

Excess alcohol or acid is required for the synthesis of monoesters or diesters of the diols, respectively. The molar ratios of diol:free fatty acid (FFA) for monoesters and diesters in the reactions were 5:1 and 1:6, respectively. *Candida Antarctica* lipase B immobilized on acrylic resin (Novozyme 435) was added at 5% weight basis of excess alcohol or acid. The reaction mixture was gently stirred at 25°C for 24 hr. In order to minimize oxidation of oleic acid, reactions were done at room temperature even though 50°C was recommended as the optimum temperature for esterification catalyzed by Novozyme 435 [5]. Molecular sieve (4 Å) was added twice to remove water of esterification with a ratio of 1 g molecular sieve to 1.8 g water at 1 and 5 hr. Upon the completion of the reaction, the esters were extracted with hexane and washed with 5% of aqueous sodium bicarbonate followed by water. When the monoester was the target, centrifugation was necessary to break the emulsion and thus remove the water and alcohol since the monoester itself was an excellent emulsifier. Then the product was subjected to silica gel column chromatography (1 g silica/0.1 g oil) for further purification. The diester, which was less polar, was eluted with 5% diethyl ether in hexane, and monoester was eluted with 15% diethyl ether in hexane.

*p*-Toluenesulfonic acid (*p*-TSA) was also used for producing of diol esters with benzene reflux to remove water of esterification. High yield, 88% for ethylene glycol dioleate, for example, could be achieved in a short time. However, *p*-TSA esters were also produced. Similar findings were reported by Jiang and Hammond [6]. The *p*-TSA esters were difficult to remove from the target esters. Thus, all the diol esters used for physical properties measurements were prepared by enzymatic methods.

*Syntheses of Pentaerythritol Esters*

The synthesis of pentaerythritol (PET) tetraoleate was completed with *p*-TSA as catalyst. About 2% *p*-TSA (of total weight of reactants) was added to a 150-ml two-neck round-bottom flask containing 50 ml benzene. The flask was connected to a condenser via a 4-ml Dean-Stark trap. After the water in *p*-TSA was transferred to the trap, oleic acid (10 mM) and PET (2 mM) were added with stirring and refluxed 6-8 hr or until ~0.15 ml water was collected in the trap.

The synthesis of the PET trioleate was catalyzed by Novozyme 435 at 50°C. Oleic acid (10 mM), PET (2 mM) and 5% enzyme (w/w based on alcohol) were mixed with stirring for 32 h.

Upon the completion of the reaction, PET esters and unreacted FFA were extracted with hexane and washed with 2% aqueous acetic acid and then water. The FFAs were removed by applying the products in hexane to an anion exchange column filled with Amberlite A21, which was weakly basic, macroreticular anion-exchange resin. The column was preconditioned with water (x2) and hexane before use. Then the mixture of reaction products dissolving in hexane was applied to the column at a ratio of 0.9 g oil/10 ml resin and flew by gravity. After the neutral oil was collected, the FFA retained in the column could be washed with 10% sulfuric acid. The column was regenerated by flushing with 4% aqueous sodium hydroxide and followed by water [7]. The PET tetraoleate and trioleate esters were separated from other impurities with a silica gel column using an increasing gradient of a mixture of diethyl ether and hexane (5%-20%).

The products were monitored with TLC. Hexane–diethyl ether–acetic acid (70:30:1) was used as the developing solvent. The separation was visualized under ultraviolet light after spraying with 0.1% (w/v) 2', 7'-dichlorofluorescein in methanol. Methyl oleate, oleic acid, tristearin, distearin and monopalmitin were used as standards.

*Instrument Analysis*

Melting points of the synthesized esters were measured with a DSC7 differential scanning

calorimeter equipped with an Intracooler System I (Perkin Elmer, Norwalk, CT, USA). The temperature programs and calibrations with indium and decane were described previously [8].

The viscosities of the various esters were determined in the temperature range 0 - 80°C with a Brookfield DV II + viscometer (Brookfield Inc., Stoughton, MA, USA) using a CP42 cone spindle. The instrument condition, temperature calibration, and procedure for measurements were described previously [8].

GC analysis was conducted on a HP5890 Series II instrument (Hewlett-Packard, PA, USA) with a SPB-1 fused silica column (15 m x 0.25 mm x 0.25 μm) (Supelco, Bellefonte, PA, USA). The injector and flame detector were at 300°C, and the oven temperature was programmed from 100 to 300°C at a rate of 10°C/min. Temperature was then held at 300°C for 10 min.

The structures of the esters in $CDCl_3$ were confirmed by $^{1}H$ NMR and $^{13}C$ NMR with a Varian VXR-400 NMR spectrometer (Varian Inc., Palo Alto, CA, USA).

## Results and discussion

### Syntheses

The diol and polyol esters that were synthesized are shown in Table 1 along with the abbreviated names that are used throughout the paper.

The esterification of FFAs with ethylene glycol preferentially formed diester whereas those with 1,2-propanediol and 2,3-butanediol had a strong tendency to form monoesters even when excess acids were added in the reaction. As a result, the yield of EGDO, PGMO and BDMO were above 90%. The yields of EGMO, PGDO, PGD15a, and BDDO averaged 40-50%. The 2,3-butanediol used was a mixture of racemic and meso forms. GC results for BDDO and BDMO using a SPB-1 column showed double peaks for both, which suggested that the products were mixtures of isomers.

Two isomers of PGMO were obtained. The double GC peaks that were obtained were distinguished using $^{1}H$ NMR bands at 5.060, 4.093, and 3.710 ppm. The NMR data indicated that the reaction preferred esterification at the 1-position of 1,2-propanediol. The GC

percentages for the two isomers indicate that the 1-position was favored over the 2- position by 68.8 to 31.2%.

The reaction between 2,3-butanediol and ricinoleic acid was achieved with an 88% yield of BDMR produced at room temperature. GC showed two isomers in a ratio of 47.4 to 41.2. The product BDMR was very viscous as shown in Table 2. Thus, heating the reaction mixture to 60°C allowed better mixing and improved the yield to 93% and favored one isomer over the other by 65.7 to 28.4%. The remaining 7% includes the diol and FFA. FFA was removed by ion exchange column and the diol was removed by distillation at 0.1 Torr.

The products of PET ester synthesis could not be analyzed by GC because of their large molecular weights and low volatility, so they were examined by TLC. The PET tetraoleate preparation had low yield when catalyzed by enzyme. Using *p*-TSA as catalyst improved the yield considerably. The product was a mixture of FFA and PET esterified to different extents as shown by TLC (Fig. 1). Free pentaerythritol was removed by extracting with water. FFA was removed by an ion-exchange column as described earlier. Neutralization with sodium carbonate was avoided during the purification of PET trioleate to avoid stable emulsions. The eluting fractions from a silica gel column were spotted on a TLC plate. Fig. 1 shows the fractions obtained by hexane with various concentrations of diethyl ether. In the column showing the unfractionated starting materials, PET tetraoleate migrated to the highest position followed by FFA, PET trioleate, PET dioleate and PET monooleate. The intensities of the tetraoleate and FFA were greater than those of the other esters in Fig. 1. According to Eychenne et al [9], the hydroxy group of the triester is less available, and consequently, less reactive than the hydroxy groups of the mono- and diesters. So the shift of the equilibrium from the triester to the tetraester was slower than that for the other partial esters. This accounted for the considerable amounts of trioleate present in the end product even after 10 hr of refluxing. The pentaerythritol used in the synthesis seems to have a number of impurities in small amounts. Some of these seemed to be increased by treatment with *p*-TSA. Careful chromatography allowed us to obtain the PET tetraoleate that was free of extraneous spots.

The PET trioleate was obtained from a Novozyme 435-catalyzed reaction. The reaction conditions greatly affected the conversions of PET triester. At 25°C, there were

hardly any conversions to the trioleate. Increasing reaction temperature to 50°C reduced the reaction mixture viscosity, and the improved mixing provided much better yields. Molecular sieves were used to remove water and drive the reaction. After 32 hr, considerable amounts of PET trioleate were obtained as shown by TLC, and a fraction was obtained with only one band shown on TCL.

Melting Points

The melting points of all the samples are shown in Table 1. The monoester of each diol and polyol had lower melting point than its diester, even though the monoester had a free hydroxyl group, which would increase the melting point [10]. The smaller MW of monoesters, which was about half of the corresponding diesters, might account for the low melting points.

Comparing the three types of diol monoesters, BDMO had the lowest melting point at -48.6°C, PGMO was the second at -11.8°C, and EGMO was the highest at -2.1°C. This is in line with our expectation that ester groups with more branches would be more difficult to closely pack in a crystal, and thus, have lower melting point. However, PGDO had the lowest melting point among the three types of diesters. PGD15a melted at 9.1°C. Its reaction precursor, 12-methyltetradecanoic acid methyl ester (anteiso-15) melted at -9°C [11]. When anteiso-15 was esterified with 1,2-propanediol, the resultant esters were still saturated and had methyl branches at both ends and in the middle of the molecule. We expected that PGD15a would have a low melting point, but it did not.

BDM10 and BDD10 melted at -10.7 and 31.8°C, respectively, much higher than BDMO and BDDO. Considering methyl decanoate melts about 4°C higher than methyl oleate, it was not unexpected to see that decanoate esters would melt higher than the oleate esters. The reason for BDMO's low melting point is not clear. It may be because it is a mixture of isomers. Replacing oleate with ricinoleate in BDMR resulted in a compound that did not crystallize, at least in the temperature range that we studied.

PET tetraoleate and trioleate melted at -10.4 and -14.1 °C, respectively.

Melting Profiles

BDMO synthesized in this study was a mixture of optical isomers but only showed one broad peak on the DSC curve. The melting temperature for each of its pure forms should be further studied since only one isomer would be likely if this synthesis were carried out in an oil seed.

BDDO, which showed double peak on GC, also had two melting peaks on the DSC curve (Fig. 2). Manipulating the temperature program did resolve them into one peak, so they did not appear to be polymorphic forms. They may represent a racemic mixture and a meso form. The two isomers of BDDO melted at -6.0 and 1.9°C. Similarly, BDD10 showed two melting peaks at 27.0 and 31.8°C (Fig. 3). The isomeric peaks of BDM10 melted at -14.3 and -10.7°C, respectively (Fig. 4).

The melting profile of PET trioleate showed polymorphism (Fig. 5). When the sample was heated from -60 to -5°C, multiple melting peaks appeared, and the multiple peaks could be resolved to a single peak by changing the cooling processes. The temperature of the single peak, which was also the highest melting form in the polymorphism, was reported as the melting point.

The melting profiles of esters that only had a single melting peak are not shown.

Viscosity

The viscosities of diol and polyol esters are shown in Table 2. Regardless of the structures of the esters, viscosities all decrease with the increase of temperatures. Most of the diol esters that were derived from oleic acid showed similar viscosities and there was little difference in viscosity between the corresponding monoester and diester except that BDM10 had about 3.5 cp higher viscosity at 35°C than its diester (BDD10). The extra hydroxy group on monoesters enhanced the intermolecular attractions by H-bonding, but this was almost exactly counterbalanced by the additional oleate moiety in the oleate diesters. In the shorter decanoate diesters, the extra 10-carbon chain was not able to counterbance the increase in viscosity caused by the hydroxy group in the monoester. Both BDM10 and BDD10 had

lower viscosities than other diol esters due to their shorter chain lengths in their ester moieties.

The variations of viscosities with temperatures were calculated by Eq. 1 [12, 13].

$$\eta = \eta_0 e^{\beta\left(\frac{1}{T}-\frac{1}{T_0}\right)} \quad (1)$$

where $\eta$ and $\eta_0$ are the viscosity (cp) at temperature (°C) $T$ and reference temperature $T_0$, respectively, and $\beta$ is the temperature-viscosity coefficient. The higher value of $\beta$ indicates larger changes of viscosity with temperature. The $\beta$ for all the esters at temperatures between 35 and 80°C are shown in Table 2. Most of the diol diesters had lower values than the corresponding diol monoesters. PET tetraoleate had smaller viscosity variation than PET trioleate. This is in line with Basu et al[3]. The free hydroxy group in monoesters increases the oil viscosity by intermolecular H-bonding. But the weak H-bond is not stable so that such intermolecular bindings will be lost at high temperature and oil viscosity decreases dramatically. Thus, the $\beta$ value of the monoesters of diols and the triester of PET were greater than those of the corresponding fully esterified compounds. BDMR, which had two free hydroxy groups, had the largest $\beta$ value.

NMR

The featured chemical shifts in $^{1}$H NMR and $^{13}$C NMR of synthesized compounds were shown as follows.

EGDO, $^{1}$H NMR: δ 5.381 (4H, m, –C**H**=C**H**−), 4.275 (4H, s, COOC**H**$_2$C**H**$_2$OOC), 2.326 (4H, t, α to carboxyl), ~1.970 (8H, m, α to double bonds), 1.624 (4H, m, β to carboxyl), ~1.265 (40H, m, methylene envelop), 0.962 (6H, t, protons on terminal carbons);

PGDO, $^{1}$H NMR: δ 5.394 (4H, m, –C**H**=C**H**−), 5.182 (1H, m, COOC**H**(CH$_3$)CH$_2$OOC), 4.202-4.105 (2H, m, COOCH(CH$_3$)C**H**$_2$OOC), 2.340 (4H, m, α to carboxyl), 2.067 (8H, m, α to double bonds), 1.663 (4H, m, β to carboxyl), ~1.351 (43H, m, methylene envelop and COOCH(C**H**$_3$)CH$_2$OOC), 0.931 (6H, t, protons on terminal carbons);

PGMO (free –OH at 1-position), $^{1}$H NMR: δ 5.25 (2H, m, C9 –C**H**=C**H**−), 5.060 (1H, m, COOC**H**(CH$_3$)CH$_2$OH), 4.093 (2H, m, COOCH(CH$_3$)C**H**$_2$OH), 2.421 (3H, m, C2 −C**H**$_2$ and

COOCH($CH_3$)$CH_2$O**H**), 2.082 (4H, m, C8−C**H**$_2$ and C11 −C**H**$_2$), 1.707 (2H, m, C3 −C**H**$_2$), ~1.358 (23H, m, C4-7 and C12-17 and COOCH(C**H**$_3$)$CH_2$OOC), 0.952 (3H, t, C18−C**H**$_3$);

PGMO (free –OH at 2-position), $^1$H NMR: δ 5.25 (2H, m, C9 –C**H**=C**H**−), 4.093 (2H, m, COOC**H**$_2$CH($CH_3$)OH), 3.710 (1H, m, COOC**H**$CH_2$($CH_3$)OH),2.421 (3H, m, C2 −C**H**$_2$ and COOCH$CH_2$($CH_3$)O**H**), 2.082 (4H, m, C8−C**H**$_2$ and C11 −C**H**$_2$), 1.707 (2H, m, C3 −C**H**$_2$), ~1.358 (23H, m, C4-7 and C12-17 and COOCH$CH_2$(C**H**$_3$)OH), 0.952 (3H, t, C18−C**H**$_3$);

PGD15a, $^1$H NMR: δ 5.147 (1H, m, COOC**H(**$CH_3$)$CH_2$OOC), 4.159-4.062 (2H, m, COOCH($CH_3$)C**H**$_2$OOC), 2.296 (4H, m, α to carboxyl), 1.618 (6H, m, β to carboxyl and terminal carbons $CH_3CH_2$($CH_3$)C**H**$CH_2$), ~1.264 (35H, m, methylene envelop, terminal carbons $CH_3$C**H**$_2$($CH_3$)$CHCH_2$ and terminal carbons COOCH(C**H**$_3$)$CH_2$OOC), 1.12 (4H, m, terminal carbons $CH_3CH_2$($CH_3$)CHC**H**$_2$), 0.973 (impurity from PGD16i), 0.89 (6H, t, terminal carbons $CH_3CH_2$(C**H**$_3$)$CHCH_2$), 0.859 (6H, t, protons on terminal carbons C**H**$_3CH_2$($CH_3$)$CHCH_2$);

BDDO, $^1$H NMR: δ 5.379 (4H, m, –C**H**=C**H**−), 5.027 (2H, m, COOC**H**($CH_3$)C**H**($CH_3$)OOC), 2.330 (4H, m,α to carboxyl), 2.054 (8H, m, α to double bonds), 1.649 (4H, m, β to carboxyl), ~1.320 (46H, m, methylene envelop and COOCH(C**H**$_3$)CH(C**H**$_3$)OOC), 0.918 (6H, t, protons on terminal carbons);

BDMO, $^1$H NMR: δ 5.390 (2H, m, C9 –C**H**=C**H**−), 4.812 (1H, m, COOC**H**($CH_3$)CH($CH_3$)OH), 3.795 (1H, m, COOCH($CH_3$)C**H**($CH_3$)OH), 2.365 (3H, m, C2 −C**H**$_2$ and COOCH($CH_3$)$CH_2$($CH_3$)O**H**), 2.065 (4H, m, C8−C**H**$_2$ and C11 −C**H**$_2$), 1.676 (2H, m, C3 −C**H**$_2$), ~1.335 (26H, m, C4-7 and C12-17 and COOCH(C**H**$_3$)CH(C**H**$_3$)OH), 0.928(3H, t, C18−C**H**$_3$);

BDD10, $^1$H NMR: δ 5.068 (2H, m, COOC**H**($CH_3$)C**H**($CH_3$)OOC), 2.365 (4H, m, α to carboxyl), 1.689 (4H, m, β to carboxyl), ~1.342 (30H, m, methylene envelop and COOCH(C**H**$_3$)CH(C**H**$_3$)OOC), 0.958 (6H, t, protons on terminal carbons);

BDM10, $^1$H NMR: δ 4.814 (2H, m, COOC**H**($CH_3$)CH($CH_3$)OH), 3.799 (1H, m, COOCH($CH_3$)C**H**($CH_3$)OH), 2.369 (2H, m, C2 −C**H**$_2$), 2.236 (COOCH($CH_3$)CH($CH_3$)O**H**), 1.676 (2H, m, C3 −C**H**$_2$), ~1.314 (18H, m, C4-9 and COOCH(C**H**$_3$)CH(C**H**$_3$)OH), 0.929(3H, t, C10−C**H**$_3$);

BDMR, $^{1}$H NMR: δ 5.518 (2H, m, C9 –C**H**=C**H**−), 4.805 (1H, m, COOC**H**($CH_3$)CH($CH_3$)OH), 3.787 (1H, m, COOCH($CH_3$)C**H**($CH_3$)OH), 3.653 (1H, m, C12-O**H**), 2.362~2.090 (7H, m, C2 −C**H**$_2$, COOCH($CH_3$)CH($CH_3$)O**H**, C8-C**H**$_2$ and C11-C**H**$_2$), 1.668 (2H, m, C3 −C**H**$_2$), ~1.351 (24H, m, C4-7 and C13-17 and COOCH(C**H**$_3$)CH(C**H**$_3$)OH), 0.926(3H, t, C18−C**H**$_3$);

PET trioleate, $^{1}$H NMR: δ 5.379 (6H, m, –C**H**=C**H**−), 4.144 (6H, s, HO($CH_2$)C(C**H**$_2$)$_3$), 3.519 (2H, m, HO(C**H**$_2$)C($CH_2$)$_3$), 2.360 (6H, m, α to carboxyl), 2.053 (12H, m, α to double bonds), 1.646 (6H, m, β to carboxyl), 1.335 (60H, m, methylene envelop), 0.916 (9H, t, protons on terminal carbons); $^{13}$C NMR: δ 174.613 (3C, C1), 130.661 (6C, –**C**H=**C**H−), 62.774 (3C, HO($CH_2$)C(**C**H$_2$)$_3$), 61.508 (1C, HO(**C**H$_2$)C($CH_2$)$_3$, 34.981~23.538 (42C, methylene envelop), 14.979 (3C, terminal carbons).

PET tetraoleate, $^{1}$H NMR: δ 5.427 (8H, m, –C**H**=C**H**−), 4.193 (8H, s, C(C**H**$_2$)$_4$), 2.385 (8H, m, α to carboxyl), 2.094 (16H, m, α to double bonds), 1.680 (8H, m, β to carboxyl), 1.365 (80H, m, methylene envelop), 0.965 (12H, t, protons on terminal carbons); $^{13}$C NMR: δ 174.337 (4C, C1), 130.982 (8C, –**C**H=**C**H−), 63.268 (1C, **C**($CH_2$)$_4$), 35.240~23.871 (56C, methylene envelop), 15.307 (4C, terminal carbons).

**Acknowledgement**

We thank the Plant Science Institute and Center for Crop Utilization Research at Iowa State University for support.

**References**

1. Lal K, Carrick V (1994) Performance testing of lubricants based on high oleic vegetable oils. Synth Lubr 11: 189-206

2. Randles SJ (1999) Esters. In: Rudnick LR, Shubkin RL (eds) SyntheticLubricants and High-Performance Functional Fluids. Marcel Dekker, Inc. New York, pp 67

3. Basu HN, Robley EM, Norris ME (1994) Preparation of glycol derivatives of partially hydrogenated soybean oil fatty acids and their potential as lubricants. J Am Oil Chem Soc 71: 1227-1230

4. Schneider MP (2006) Plant-oil-based lubricants and hydraulic fluids. J Sci Food

Agric 86: 1769-1780

5. Roenne, TH, Xu X, Tan T (2005) Lipase-catalyzed esterification of lactic acid with straight-chain alcohols. J Am Oil Chem Soc 82: 881-885

6. Jiang YJ, Hammond EG (2002) Toluenesulfonate-acyl esters of ethylene glycol and other 1,2-diols as industrial antioxidants with cupric ion. J Am Oil Chem Soc 79: 791-796

7. Schoenenberger KA, Fries W (1978) Removal of free fatty acids from water immiscible fluids via ion exchange resins. US Patent 4,125,550

8. Yao L, Hammond EG, Wang T (2008) Melting points and viscosities of fatty acid esters that are potential targets for engineered oilseed. J Am Oil Chem Soc 85: 77-82

9. Eychenne V, Mouloungui Z, Gaset A (1998) Total and partial erucate of pentaerythritol. Infrared spectroscopy study of relationship between structure, reactivity, and thermal properties. J Am Oil Chem Soc 75:293-299

10. Yao L, Hammond EG, Wang T (2009) Melting points and viscosities of ricinoleate esters that are potential biolubricant targets for engineered oilseed. J Am Oil Chem Soc. In preparation

11. Yao L, Hammond EG (2006) Isolation and melting properties of branched fatty esters from lanolin. J Am Oil Chem Soc 83:547–552

12. Roelands CJA (1966) Correlation aspects of the viscosity-temperature-pressure relationship of lubricating oils, Ph.D. thesis, Delft University of Technology, Netherlands

13. Bushan B (2002) Introduction to Tribology. John Wiley & Sons, Inc, New York. pp 430

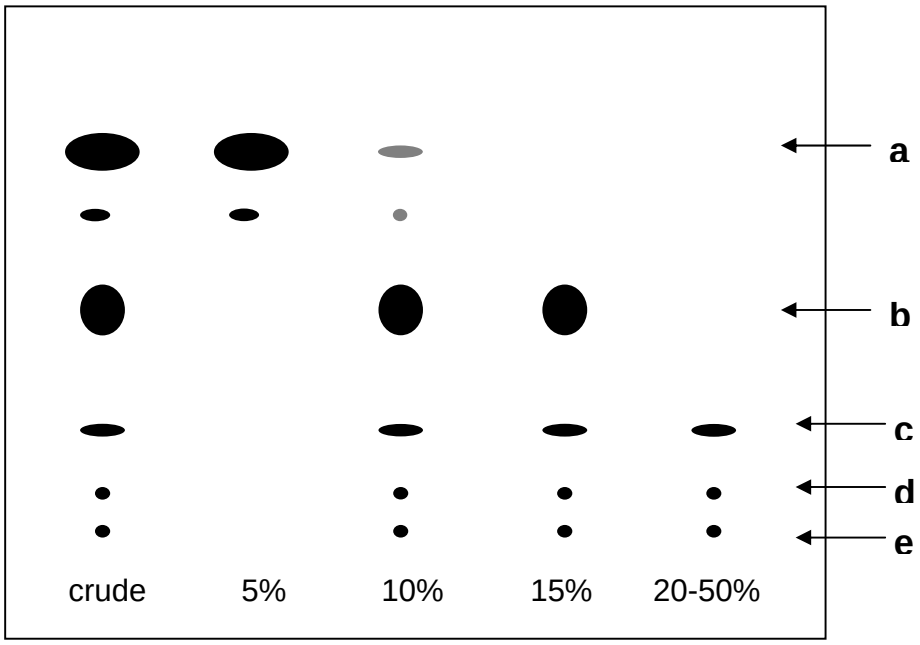



**Fig. 1** Thin layer chromatography of PET tetraoleate reaction products and elutes from silica gel column chromatography. Crude – starting materials before TLC;t he eluting solvent ratios 5, 10, 15, 20-50% are used for naming the fractions, with 5% means diethyl ether/hexane (5:95, v/v); a – PET tetraoleate; b – FFA; c – PET trioleate; d – PET dioleate; e – PET monooleate

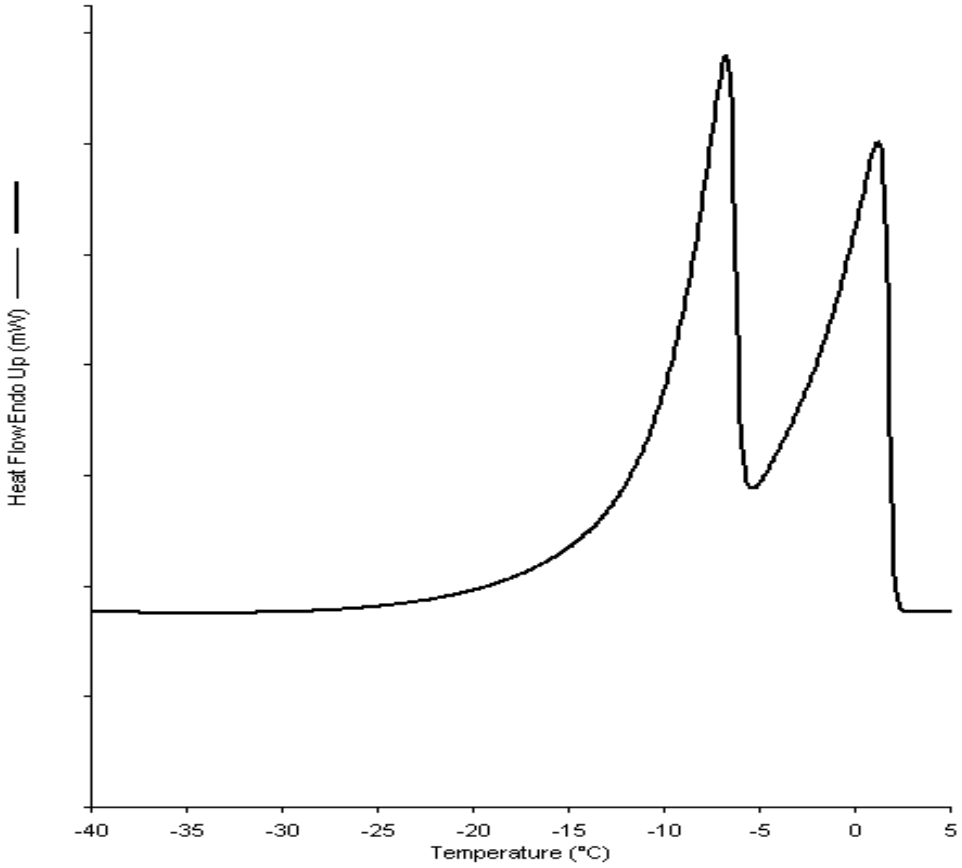



**Fig. 2** Melting profile of 2,3-butanediol dioleate. — Heat from -60 to 20°C at 5°C /min

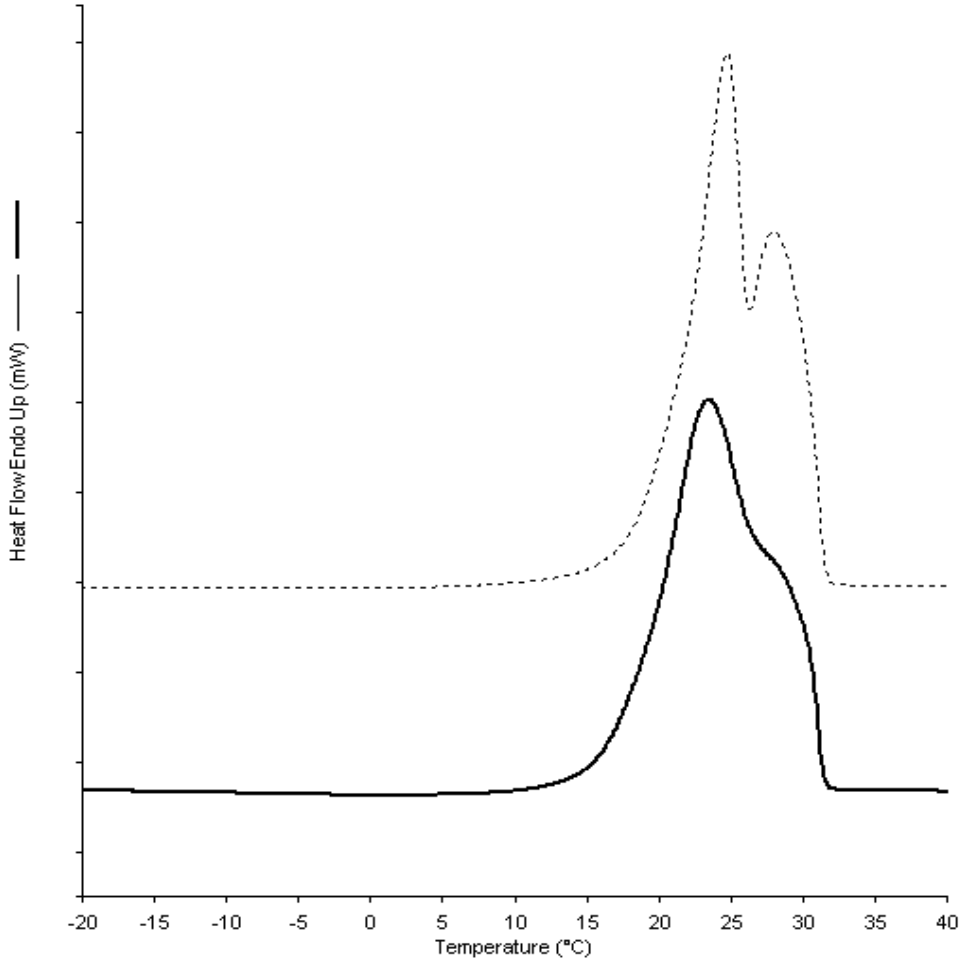



**Fig. 3** Melting profile of 2,3-butanediol didecanoate. — Heat from -20 to 25°C at 5°C /min, hold at -20°C for 7 min, cool to -20°C at 10°C/min, heat from -20 to 40°C at 5°C/min; ··· Heat from -20 to 40°C at 5°C/min

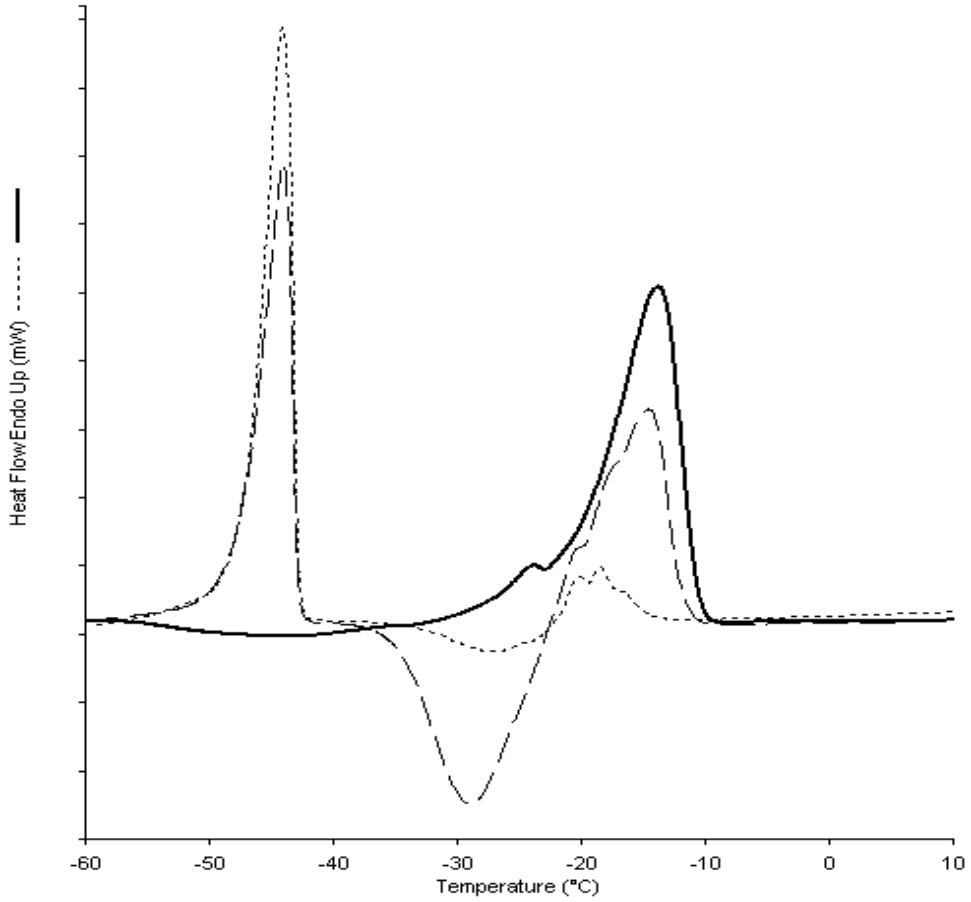



**Fig. 4** Melting profile of 2,3-butanediol monodecanoate. — Heat from -60 to -20°C at 5°C /min, hold at -20°C for 7 min, cool to -60°C at 10°C/min, heat from -60 to 10°C at 5°C/min; --- ··· Heat from -60 to 10°C at 5°C/min

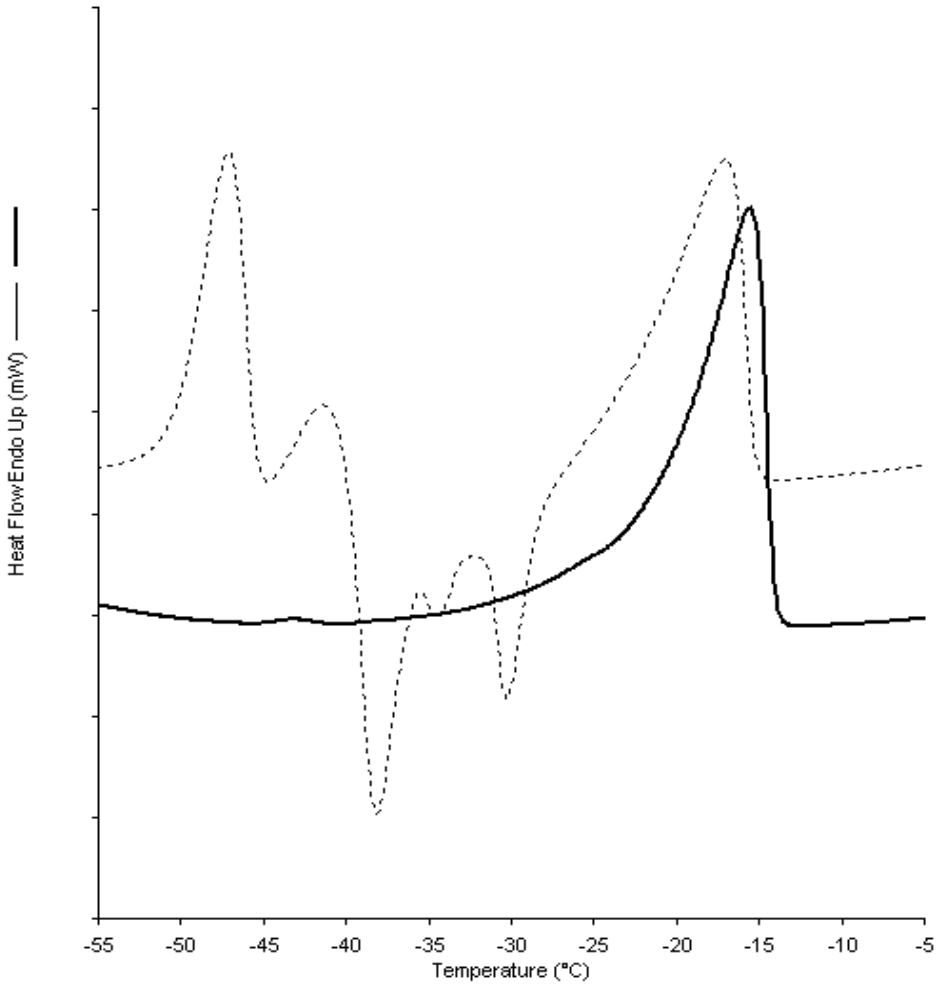



**Fig. 5** Melting profile of pentaerythritol trioleate. — Heat from -60 to -20°C at 5°C /min, hold at -20°C for 7 min, cool to -60°C at 20°C/min, heat from -60 to 0°C at 5°C/min; ··· Heat from -60°to 0°C at 5°C/min

**Table 1** Melting points of various diol and polyol esters

| **Fatty esters** | **Abbr.** | **m.p. *(°C)** | |
|---|---|---|---|
| Ethylene glycol dioleate | EGDO | | 8.5 |
| Ethylene glycol monooleate | EGMO | | -2.1 |
| 2,3-Butanediol dioleate* | BDDO | -6.0 | 1.9 |
| 2,3-Butanediol monooleate | BDMO | | -48.6 |
| 1,2-Propanediol dioleate | PDDO | | -7.4 |
| 1,2- Propanediol monooleate | PDMO | | -11.8 |
| 1,2- Propanediol di-anteiso-15 | PDD15a | | 9.1 |
| 2,3-Butanediol didecanoate* | BDD10 | 27.0 | 31.8 |
| 2,3-Butanediol monodecanoate* | BDM10 | -14.3 | -10.7 |
| 2,3-Butanediol monoricinoleate | BDMR | | - |
| Pentaerythritol tetraoleate | PET tetraoleate | | -10.4 |
| Pentaerythritol trioleate | PET trioleate | | -14.1 |

*

*Melting points of isomers are presented

**Table 2** Viscosities (cp) of diol and polyol esters at various temperatures

| Temp (°C) | Viscosity (cp) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | EGDO | PGDO | PGMO | PGD15a | BDDO | BDMO | BDD10 | BDM10 | BDMR | PET tetraoleate | PET trioleate |
| -5 | | 142.8 | 172.3 | | 150.0 | 237.4 | | 126.9 | | | |
| 0 | | 107.3 | 125.3 | | 113.4 | 167.0 | | 88.6 | 3293 | 417.9 | 602.5 |
| 5 | | 82.3 | 91.7 | | 87.0 | 118.5 | | 63.7 | 1896 | 305.9 | 426.9 |
| 10 | | 64.3 | 68.7 | 67.0 | 67.5 | 87.4 | | 46.6 | 1169 | 227.0 | 305.3 |
| 15 | | 50.6 | 52.5 | 52.2 | 53.1 | 64.7 | | 35.1 | 731.1 | 171.8 | 232.5 |
| 20 | | 40.6 | 40.8 | 41.3 | 42.3 | 49.2 | | 26.8 | 467.9 | 132.1 | 174.0 |
| 25 | | 32.9 | 32.9 | 33.1 | 34.9 | 38.7 | | 20.9 | 309.6 | 106.3 | 132.7 |
| 30 | | 27.1 | 26.2 | 27.2 | 28.5 | 30.3 | | 16.4 | 213.6 | 84.5 | 103.4 |
| 35 | 20.6 | 22.6 | 21.3 | 22.4 | 23.7 | 24.3 | 9.7 | 13.2 | 150.4 | 68.0 | 81.3 |
| 40 | 18.0 | 19.0 | 17.3 | 18.7 | 19.9 | 19.7 | 8.4 | 10.7 | 107.0 | 55.8 | 65.0 |
| 45 | 15.7 | 16.2 | 14.4 | 15.8 | 17.0 | 16.1 | 7.3 | 8.8 | 78.7 | 46.0 | 52.8 |
| 50 | 13.9 | 13.9 | 12.2 | 13.5 | 14.6 | 13.4 | 6.3 | 7.4 | 58.7 | 38.6 | 43.6 |
| 55 | 12.3 | 12.1 | 10.3 | 11.7 | 12.6 | 11.2 | 5.6 | 6.2 | 44.5 | 32.5 | 36.2 |
| 60 | 10.9 | 10.6 | 8.8 | 10.1 | 11.0 | 9.5 | 4.9 | 5.3 | 34.5 | 27.7 | 30.4 |
| 65 | 9.7 | 9.4 | 7.6 | 8.9 | 9.7 | 8.1 | 4.4 | 4.6 | 27.3 | 23.8 | 25.8 |
| 70 | 8.6 | 8.3 | 6.6 | 7.9 | 8.6 | 7.0 | 3.9 | 4.0 | 21.8 | 20.7 | 22.1 |
| 75 | 7.7 | 7.4 | 5.8 | 7.0 | 7.6 | 6.1 | 3.5 | 3.5 | 17.7 | 18.0 | 19.2 |
| 80 | 7.0 | 6.6 | 5.1 | 6.3 | 6.9 | 5.4 | 3.2 | 3.1 | 14.5 | 15.9 | 16.8 |
| $\beta$ | 67.2 | 76.6 | 89.0 | 78.9 | 76.8 | 93.6 | 69.0 | 90.2 | 145.6 | 90.4 | 98.1 |

# CHAPTER 5. BOUNDARY LUBRICATION PROPERTIES OF FATTY ACID DERIVATIVES AS POTENTIAL BIOLUBRICANTS

A paper to be submitted to
*The Journal of American Oil Chemists' Society*

Linxing Yao[1,3], Satyam Bhuyan[2,4], Earl G. Hammond[1,5], Tong Wang[1]
Sriram Sundararajan[2]

**Abstract** A series of fatty ester were previously synthesized as potential biolubricants, and some of them had appropriate melting points and viscosities and acceptable stability against oxidation. In this study， the boundary lubricities of some of these fatty esters were tested. The esters tested include oleate, ricinoleate and mono 2, 3-butanediol esters. Microscale friction tests were utilized to evaluate their boundary lubrication behavior. Long-chain linear ricinoleate esters and 2,3-butanediol monooleate showed considerable improvements in lubricity compared to a typical mineral oil base. Methyl 12-decanoylricinoleate, which has a long 10-carbon branch showed an antifriction effect comparable to straight chain methyl ricinoleate. Methyl 12-acetylricinoleate had significantly better antifriction effect than methyl ricinoleate. 2,3-Butanediol monoricinoleate exhibited higher friction than its nonhydroxy counterpart, 2,3-butanediol monooleate. Oleate esters showed poorer lubricity as the chain length of their alcohol moiety increased. The tested fatty esters showed promise for improving the lubricity of the base oil. A possible explanation of the lubricity effects based on molecular packing of these compounds on metal surfaces was proposed.

---

[1]Graduate student, University Professor Emeritus, and Associate Professor, respectively, Department of Food Science and Human Nutrition, Iowa State University.
[2]Graduate student, and Associate Professor, respectively, Department of Mechanical Engineering, Iowa State University.
[3]Primary researcher and author.
[4]Primary researcher
[5]Author for correspondence

**Keywords** biolubricant · boundary lubrication · fatty esters · friction · microtribometer · oleate · 2,3-butanediol · ricinoleate · wear

## Introduction

Petroleum-based lubricants have been criticized recently because of environmental concerns based on their poor biodegradabilities and toxicities. In addition, increasing oil prices have encouraged the development of substitutes from renewable sources. Conventional vegetable oils, which are mainly triacylglycerols, have advantages for use as lubricants because they provide excellent boundary lubrication, high viscosity indices, high flash points and low volatilities [1]. However, poor low-temperature performance and tendency to oxidize greatly limit their applications. Synthetic esters can exhibit better performance than typical vegetable oils but often at much higher costs. Various ideas have been proposed to improve the properties of conventional vegetable oils, such as by chemical [2] and genetic modifications [3]. Thus, it is important to identify fatty acid derivatives with suitable lubrication properties and document their properties to provide guidance in the development of biolubricants from renewable oils. So we have synthesized various fatty acid derivatives and characterized their melting points and viscosities. Methyl and isopropyl esters of fatty acids with iso- or anteiso-methyl branches and with acid chain length 13 to 18 have considerably lower melting points than their straight-chain counterparts because the methyl branch prevents close chain packing in crystals [4]. A study on the boundary lubricity of methyl-branched fatty acid methyl esters showed that they provided excellent protection on metal surfaces against friction [5]. Oleate esters derived from oleic acid and various chain lengths of alcohols melted from -16 to -33°C [6]. Both methyl-branched fatty acid methyl esters (FAMEs) and oleate esters had viscosities around 10~30 cp at 0°C and 2 cp at 70°C. Ricinoleate esters had excellent melting points as well as viscosities. Ricinoleate esters melted from -3 to -29°C and have viscosities of ~130 to 180 cp at -5°C and 4 cp at 80°C. Methyl 12-acetylricinoleate resisted crystallization and became gel-like solid at temperature as low as -50°C. Methyl 12-decanoylricinoleate melted at -14°C [7]. 2,3-Butanediol monooleate melted at -48°C and had viscosity of 167 cp at 0°C and 5 cp at 80°C. 2,3-Butanediol monodecanoate, a saturated compound, melted at -11°C. 2,3-Butanediol monoricinoleate had high viscosity that prevented it from crystallization [8].

All these fatty acid derivatives have reasonable stability to oxidation because they were either saturated or monounsaturated. However, few studies have been conducted about the tribological behaviors of these compounds or similar structures when they were pure.

Boundary lubrication is most important during the starting and stopping of a machine, when high loads and low speed are applied to the contact surfaces. A thin layer of lubricant molecule absorbs onto the metal surfaces and provides some protection against wear [9]. Since the film cannot completely separate the two metal surfaces, there is true contacts at the surfaces' asperities. Thus, friction and wear occur and can result in significant costs. It is important to study boundary lubrication because it controls the life of mechanical components in contract and their relative motion. Under boundary lubrication, the physical and chemical interactions of the lubricant with metal surfaces dominate the tribological performances. The performance of lubricants under boundary conditions greatly depends on their chemical structures. In this study, boundary lubrication properties of certain synthesized fatty esters are described and their effect on lubricity and wear are compared.

## Materials and methods

### Materials

The fatty esters measured for their boundary lubricity were as follows. Four oleate esters with alcohol chain lengths 2, 3, 8, and 9, i.e. ethyl, propyl, octyl, and nonyl oleate were compared. They were obtained by esterifications of oleic acid and the corresponding alcohol using Novozyme 435 lipase as a catalyst [6]. Methyl ricinoleate (MeR) was compared with methyl 12-acetylricinoleate (MeR-Ac), methyl 12-decanoylricinoleate (MeR-10), butyl ricinoleate (BuR), and isopropyl ricinoleate (IsR). The procedure for synthesizing these ricinoleates with alkali catalysts was described in [7]. 2,3-Butanediol monooleate (BDMO) was compared with 2, 3-butanediol monodecanoate (BDM10) and 2,3-butanediol monoricinoleate (BDMR), and their preparation was reported in [8]. All compounds had purities of ~99% (based on gas chromatography (GC). The base oil for lubrication test was a

light mineral oil purchased from Fisher Scientific (Pittsburgh, PA, USA). The esters were tested as 10% (w/v) blend in the base oil.

## Methods

### *Microscale Friction and Wear Tests*

A custom-built reciprocating ball-on-flat micro-tribometer was used for friction and wear tests. A schematic of its major components and a detailed explanation of its mechanism can be found in Bhuyan et al. [5]. The friction and wear data was obtained from rubbing a spherical probe against a flat steel disk in a reciprocating linear motion. Load is applied normal to the surface after the sphere and disk were in contact. The normal and linear forces were monitored by computers. For this study, a steel disk (AISI 52100, hardness 848 VHN and elastic modulus 210 GPa) with 10 mm-thick and 50 mm in diameter was used. The surface was polished to a RMS roughness of 128 ± 22 nm. An AISI 52100 steel ball (1.2 mm radius) with RMS roughness of 4 nm was used as the probe. Both the probes and disks were cleaned with acetone in an ultrasonic bath for 15 min and dried using dry air prior to tests.

### *Friction Test*

The coefficient of friction (COF) was obtained from the slope of a plot of friction versus normal load as the load was ramped from 0.2 to 1000 mN. The probe's stroke distance was 40 mm at a speed of 1 mm/s. Under such conditions, the effect of sliding speed on friction and wear was negligible [5]. The tests were performed at 25°C and a relative humidity of 50%. Four replicate friction measurements were performed for each sample.

### *Reciprocating Sliding Wear Test*

A tungsten carbide ball (1.5 mm radius) was used for wear tests. Under a constant normal force of 800 mN, the probe was rubbed against the disk in a lateral distance of 60 mm at 5

mm/s for 500 cycles. The resultant wear track was measured with a contact profilometer (Dektak II) and contact mode atomic force microscopy (AFM) (Dimension 3100, Nanoscope IV, Veeco Instruments, Santa Barbara, CA, USA). Five locations on the three wear tracks of each compound were chosen randomly and measured for wear depths.

*Statistical Analysis*

The friction and wear date were analyzed using a SAS v. 9.1 program (SAS Institute, Inc., Cary, NC, USA). A *t* test with a 95% significance level based on a one-way analysis of variance was used to determine the significance of differences in COFs and wear depth of fatty esters derived from similar chemical structures. We assumed that the instrument had very high repeatability and variance between metals (disk and probe) was equal for the various esters. During wear tests, some measurements were lost and there were no records on subsampling, i.e., from which track the measurements were done, so that unequal sample sizes were used in the statistical analysis. The root mean squared errors (RMSE) for the friction test of oleate esters, friction test of ricinoleate esters, friction test of 2,3-butanediol esters, wear test of ricinoleate esters, and wear test of 2,3-butanediol esters were 0.0035, 0.0045, 0.0026, 26.8034, 26.1832, respectively.

**Results and discussion**

The microtribomer system used simulates multiple asperity contacts that occur when two metal surfaces come into contact. It is derived from a ball-on-flat tribometer systems and hence can reliably test the boundary lubrication behavior of the lubricant [9]. Microscale friction tests are particularly beneficial when only a limited quantity of the test material is available. Very little lubricant is used, and the instrument is operated under very light loads with a small contact area. As a result, friction and wear of lightly loaded lubricant are highly dependent on the surface interactions. Fig.1 shows friction data for the base oil compared with an unlubricated surface. The base oil by itself does not provide significant lubrication to the interface. This is in agreement with past studies on pure paraffin oil [5, 10].

Effects of Chemical Structure on Friction Reduction

The COFs of the oleate esters are shown in Fig.2 (a). All the tested oleate esters showed friction reductions compared with the base oil. Usually, increasing molecular weight improves the lubricity of compounds with similar chemical structures. The longer chain molecules increase the film thickness and have stronger inter-chain molecular attractions so that they can better protect the surfaces. But the friction data of oleate esters showed that propyl and octyl oleate had higher COF than ethyl oleate. There was no significant difference between propyl and octyl oleate. Nonyl oleate had a slightly lower COF than both propyl and octyl oleate, but a higher COF than that of ethyl oleate.

The COF of ricinoleate esters obtained from friction tests are shown in Fig.2 (b). Among all the ricinoleate esters, MeR-Ac, BuR and IsR showed significant improvements in lubricity compared to the base oil. MeR and MeR-10 did not provide reduction of the friction. No significant difference in COF was observed for MeR and MeR-10 even though MeR-10 had slightly lower averaged COF than that of MeR. Branched compounds had lower packing densities comparing to their straight chain counterparts and may have resulted in weaker films and provided less protection against friction. In our study, MeR-10 had much higher molecular weight than MeR even though it was branched. So it might suggest that the increase of the overall molecular weight minimizes the adverse effect of branching on enhancing lubricity. Longer alcohol chain ricinoleates, BuR and IsR had more noticeable reduction of friction compare to methyl esters. MeR-Ac showed significant lower COF than MeR, which indicated that eliminating the hydroxyl group in the middle of the hydrocarbon chain of ricinoleic acid increased its lubrication ability. Another pair of molecules derived from 2,3-butanediol provided extra evidence on this phenomenon (see next section).

The COFs of 2, 3-butanediol monoesters are shown in Fig.2(c). All the 2,3-butanediol monoesters showed improved lubricity, and BDMO exhibited the largest reduction of friction followed by BDMR and then BDM10. BDM10 had an 8 carbon shorter chain than BDMO so that it did not provide as great protection as BDMO. BDMR had considerable higher friction than its nonhydroxy counterpart BDMO. So it suggests that a –OH group in the middle of a hydrocarbon chain decreases its lubricity. Castor oil was found by others to have less

protection against wear compared to vegetable oil without the hydroxyl group [11]. The free hydroxyl group in the diester of trimethylolpropane based on palm oil and palm kernel oil had negative effects on wear properties at high concentration [12]. However, other studies also showed that ricinoleic acid increased lubiricty as additives in diesel fuel more effectively than a non-hydroxylated fatty acid such as oleic acid [13]. Considering that the designs of these studies with controversial results were different and surface chemistry between lubricants and metal surface was extremely complicated, further studies are needed to determine the effects of hydroxyl fatty acid on the lubricity under various circumstances. Those compounds with higher COFs including MeR, MeR-10, and BDM10, also were exhibited strong oscillatory friction responses at high load (800-1000 mN, data is not shown). Their high COFs might be resulted from weaker physical bonding to the metal surfaces. Supposedly they were more easily pulled off from the surface under high load due to weaker adhesion between the molecules and metal surfaces. When the lubricating fluid was squeezed out from between the metals at high load, there was no protection against friction [1].

Effects of Chemical Structure on Wear Protection

The sliding wear test showed consistent results for these fatty esters compared to the friction test (Fig.5). Among ricinoleate esters, the compounds with longer alcohol portion, BuR and IsR provided better wear protection than short chain compound MeR. Branched chain compound MeR-10 produced similar wear depth to MeR. Among 2,3- butanediol monoesters, BDMO showed the best wear protection. Both BDM10 and BDMR had deeper wear depth than BDMO. The explanations were given in previous section. However, the comparison between BDM10 and BDMR showed difference in friction test and wear test. BDMR provided better protection against friction but worse protection against wear compared to BDM10. As described in the Methods section, friction tests were performed by gradually increasing the mechanical load from 0.2 to 1000 mN during a 40 s period; wear test was performed under a constant high load at 800 mN for a 6000 s period. It suggests that BDMR's lubricity may be reduced under high mechanical load and prolonged operation time. Wear tests of oleate esters were not done.

Theory of Molecular Packing along the Metal Surfaces

The tribological results for these synthesized biolubricant compounds suggest that a complicated mechanism may exist for lubricant molecular packing along the metal surfaces under a boundary lubrication regime, especially when the lubricant has complex chemical structure. How the various fatty esters in this study would be expected to align on a metal surface is given in Fig.4. For compounds like oleate esters (Fig. 4b and Fig. 4c), the monolayer of lubricating film is formed by their polar ester bond attaching to the metal surface via H-bonding. The long hydrocarbon chains on the acid portion line up in a parallel fashion that is maintained via inter-chain van der Waal forces. When the alcohol moiety has short chain length, such as one or two carbons, the packing shape would be just as same as that of free fatty acids (Fig. 4a). However, when the chain length of alcohol increases and is no longer negligible, the packing shape has to change to accommodate the extra "tail". The broadened space between the carbon chains may result in a loosened and weaker film. Therefore, propyl and octyl oleate showed high COFs than ethyl oleate. When the alcohol moiety was 9-carbon long, the extra "tail" began to interfere with the double bond which was located at the 9,10-position on the acid moiety of oleate ester. Thus, the lubricating film was strengthened by the intramolecular forces and as a result, more reduction on friction was observed with nonyl oleate compared to octyl oleate.

The probable molecular packing of ricinoleate esters is given in Fig. 4d - 4f. Since the hydroxy group is more polar than the ester group, the hydroxy group on the n-6 position of ricinoleate esters preferentially bonds with the metal surface. Consequently, a paralleled double-chain configuration is formed with the ester group pointing out. The alcohol portion of the ester is the "waving tail". As the chain length of alcohol increases, a thicker film is formed and hence provided better lubricity performance. Esterifying a 10-carbon branch with the hydroxyl group returns the ester group on the primary chain to the metal surface. So, the whole primary chain of MeR-10 contributes the film thickness, which is as twice as the film thickness formed by MeR. However, the presence of the branch expands the gap between two aligned molecules and thus reduces the intermolecular force between them. So, MeR and MeR-10 showed similar COFs in the tests.

As illustrated in Fig. 4g - 4i, the ester group of 2, 3-butandiol monoester bonds to the metal surface and the hydroxy group on the polar head reinforces the bonding to the metal. So the alignment of BDMO on metal surface is similar to oleate ester but perhaps stronger (Fig. 4a - 4b). With a 10-carbon acid portion, BDM10 certainly formed a thinner film even though the saturated acid chains may provide a tighter packing. The extra –OH group bends half of the carbon chain of BDMR to form double-chain configuration similar to methyl ricinoleate (Fig. 4(d)) that results from the interaction between –OH and the adjacent double bond. This chain shortening of BDMR increased COF compared to that of BDMO, its nonhydroxy counterpart.

The concentration of fatty esters in mineral oil was 10% (w/v) in this study. The results more appropriately predict the reduction of friction and wear when these esters are used as additives. Further studies should include a comparison of these fatty esters with commercial additives in enhancing lubricity of base oil. It also would be interesting to see how they would behave when used as a base oil.

**Acknowledgements**

We thank the Plant Science Institute and Center for Crop Utilization Research at Iowa State University for support and Dr. Philip Dixon for help with the statistical analyses.

**References**

1. Adhvaryu A, Erhan SZ, Perez JM (2004) Tribological studies of thermally and chemically modified vegetable oils for use as environmentally friendly lubricants. Wear 257: 359-367

2. Schneider MP (2006) Plant-oil-based lubricants and hydraulic fluids. J Sci Food Agric 86: 1769-1780

3. Murphy DJ (2002) Biotechnology and the improvement of oil crops-genes, dreams and realities. Phytochem Rev 1: 67-77

4. Yao L, Hammond EG (2006) Isolation and melting properties of branched fatty esters from Lanolin. J Am Oil Chem Soc 83:547–552

5. Bhuyan S, Sundararajan S, Yao L, Hammond EG, Wang T (2006) Boundary

lubrication properties of lipid –based compounds evaluated using microtribological methods. Tribol Lett 22:167-172

6. Yao L, Hammond EG, Wang T (2008) Melting points and viscosities of fatty acid esters that are potential targets for engineered oilseed. J Am Oil Chem Soc 85: 77-82

7. Yao L, Hammond EG, Wang T (200?) Melting points and viscosities of ricinoleate esters that are potential targets for engineered oilseed. J Am Oil Chem Soc in preparation

8. Yao L, Hammond EG, Wang T (200?) Melting points and viscosities of polyol esters that are potential targets for engineered oilseed. J Am Oil Chem Soc in preparation

9. Bushan B (2002) Introduction to Tribology. John Wiley & Sons, Inc, New York, pp 533

10. Gates RS, Hsu SM (1995) Silicon nitride boundary lubrication: effect of oxygenates. Tribol Trans 38: 607-617

11. Weller DE, Perez JM (2001) A study of the effect of chemical structure on friction and wear. Lubrication Eng 56: 20-25

12. Yunus R, Fakhru'I-Razi A, Ooi TL, Iyuke SE (2004) Lubrication properties of trimethylolpropane esters based on palm oil and palm kernel oils. Eru J Lipid Sci Technol 106:52-60

13. Geller DP, Goodrum JW (2004) Effects of specific fatty acid methyl esters on diesel fuel lubricity. Fuel 83: 2351-2356)

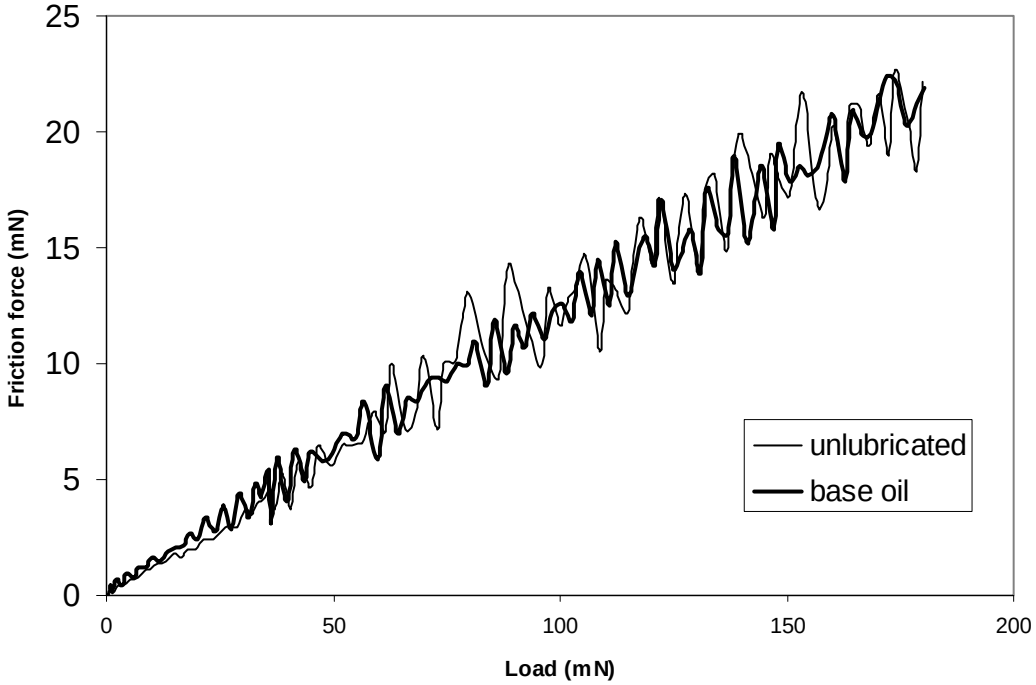



**Fig. 1** Representative friction vs. normal load plots obtained from ramped-load friction test for base oil and unlubricated surface

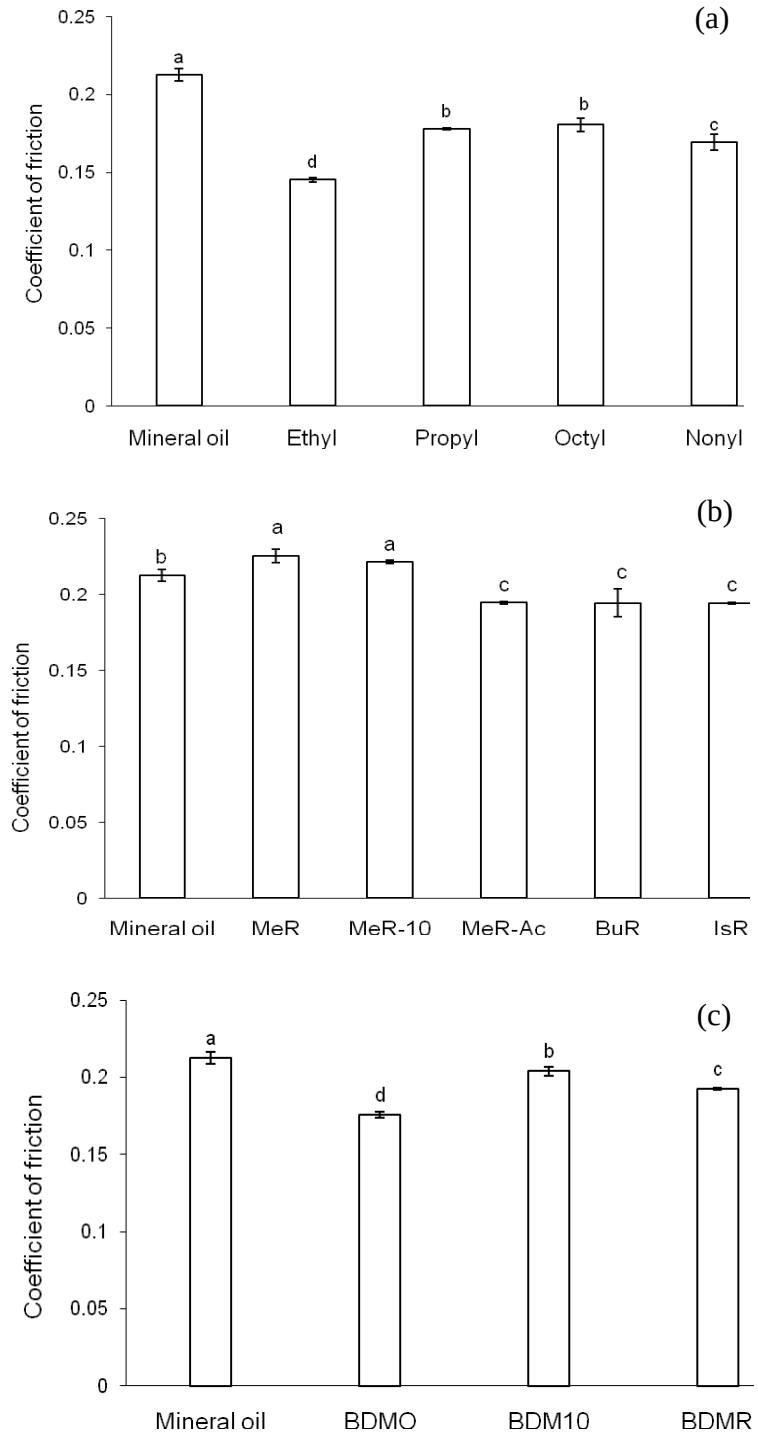



**Fig. 2** Friction test. (a) oleate esters, (b) ricinoleate esters, (c) 2,3-butanediol monoesters; different letters indicate significant difference, $P<0.05$

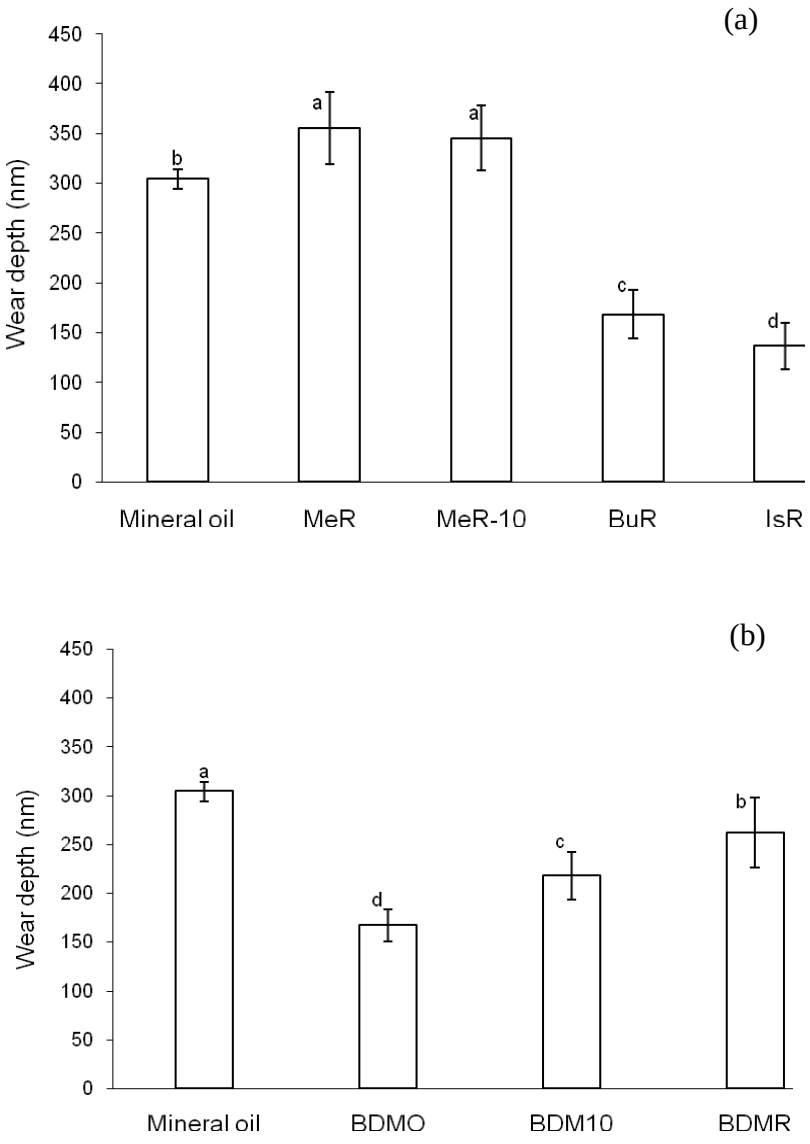



**Fig. 3** Wear test. (a) ricinoleate esters, (b) 2,3-butanediol monoester; different letters indicate significant difference, $P<0.05$

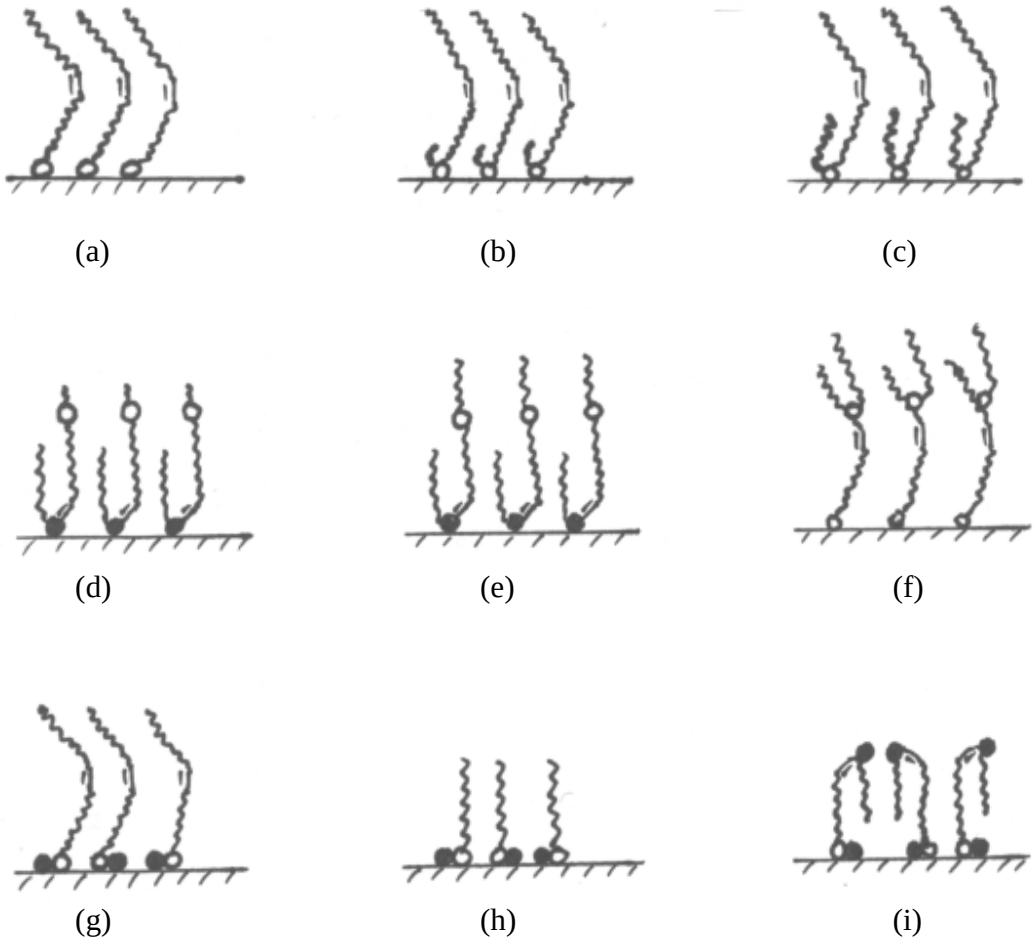



**Fig. 4** Speculations on the molecular packing of fatty esters on metal surfaces under boundary lubrication regime. Hollow circle, ester group; solid circle, hydroxy group. (a) oleic acid (b) oleate carboxylic ester with short-chain alcohol moiety, eg. ethyl oleate, (c) oleate carboxylic ester with long-chain alcohol moiety, eg. octyl oleate, (d) methyl ricinoleate, (e) ricinoleate carboxylic ester with long-chain alcohol moiety, (f) methyl 12-decanoyl ricinoleate, (g) 2,3-butanediol monooleate, (h) 2,3-butanediol monodecanoate, and (i) 2,3-butanediol monoricinoleate

# CHAPTER 6. GENERAL CONCLUSION

More than forty fatty acid derivatives were synthesized as potential biolubricant targets that might be made by genectic modifications of oilseeds or by chemical modifications of isolated fatty acids. These fatty acid derivatives varied in acid and alcohol moiety chain length, saturation, branching, and polarity. Their melting points and viscosities were measured and documented. Their boundary lubricities were compared with each other and with mineral oil. An attempt to correlate structures and properties was given.

Oleate esters of isopropanol, oleyl alcohol and normal alcohols of 1–12 carbons chain lengths were prepared by esterification with Novozyme 435 as catalyst. Isopropyl and butyl oleate have melting points of -33 and -32°C, respectively, and viscosities that range from 17 (0°C) to 2.5 cp (70°C). A decreasing melting point was observed for oleate esters with normal alcohol moieties until carbon number four, and then an increasing value. The crystallization packing of the ester group was speculated to be the cause of this phenomenon.

Ricinoleate carboxylate esters with normal alcohols of chain lengths 1 to 5 as well as the isopropyl ester were synthesized with the alkali catalysts. They melted between -3 and -29°C and had viscosities of ~130 to 180 cp at -5°C, and 4 cp at 80°C. The esterification of a branch onto the 12-hydroxyl group of ricinoleate prevents crystallization until the branch is longer than 8-carbon. Acetylated ricinoleate carboxylate esters had viscosities ranging from 49 (0°C) to 3.5 cp (80°C). Methyl and isopropyl esters of 12-acetyl- and 12-butyrylstearate melted at 4 to -15°C, which was about 50°C lower than their corresponding 12-hydroxystearate.

Mono- and diesters of diols and a polyol were synthesized enzymatically with Novozyme 435. The acyl moieties were from oleic acid, ricinoleic acid, decanoic acid. The alcohol moieties were ethylene glycol, 1,2-propanediol, 2,3-butanediol, or pentaerythritol. Polyol and diol esters that had a free hydroxyl group had lower melting points than the corresponding completely esterified polyols and diols. The completely esterified polyol and diol esters exhibited less change in viscosity with temperature than those having a free hydroxy group. Some of these compounds had melting points and viscosities that would be suitable for biolubricants, expecially 2,3-butanediol monooleate, which melted at -48.6°C and had viscosities of 19.7 cp at 40°C, and 5.4 cp at 80°C.

The fatty esters with superior melting points and viscosities were examined for their boundary lubricity. Long-chain linear ricinoleate esters, i.e. butyl and isopropyl ricinoleate, and 2, 3-butanediol monooleate showed considerable enhancement on lubricity compared to mineral oil. Long branching compound derived from methyl ricinoleate, methyl 12-decanoylricinoleate, showed comparable reduction of friction to straight chain methyl ricinoleate. Methyl 12-acetylricinoleate had significantly better antifriction effect than methyl ricinoleate. 2,3-Butanediol monoricinoleate had higher friction than its nonhydroxy counterpart, 2,3-butanediol monooleate. Oleate esters showed poorer lubricity with an increase of alcohol chain lengths. Some of the tested fatty esters show promise for improving the lubricity of base oil.

Some of the fatty esters proposed in this research were proved to have excellent physical properties and lubrication performance and suitable for uses as biolubricants produced via genetic modification or/and chemical modification. Further studies are needed to evaluate the performance of these esters when formulated with commercial products.

## ACKNOWLEDGEMENTS

It is difficult to overstate my gratitude to my supervisor Dr. Earl G. Hammond, whose guidance, help, suggestions and encouragement helped me in all the time of research and writing. I have gained invaluable experience from him. And because of him my graduate experience has been one that I will cherish forever. His personality and spirit towards science inspired me so much that he is my role model. I also want to express my sincere appreciation to Dr. Tong Wang, my Ph.D. co-advisor, for her insightful comments and stimulating ideas. This paper would not have been possible without my committee members, Drs. Lawrence A. Johnson, Basil J. Nikolau, and Philip M. Dixon, who provided valuable contributions to my writing.

I also want to thank Department of Food Science and Human Nutrition for offering me such an opportunity to study food science, the field of my most interest, and provide me a pleasant research and studying environment.

I would thank Drs. Jay-lin Jane, Pamela White for letting me use their instruments and all the member's in Drs Hammond's, Jane's, Wang's and White's lab. I greatly value their friendship and supportiveness.

I would like to express my heart-felt gratitude to my husband, Wen Zhou, whose support and care helped me overcome setbacks and stay focused on my graduate study, and to my lovely daughter, Michelle, who is always my source of joy and happiness.

Lastly, and most importantly, I would like to thank my parents, Liying Wang and Shuren Yao, for their endless support and love.