Accessing Monomers, Surfactants, and the Queen Bee Substance by Acrylate Cross-Metathesis of Long-chain Alkenones

Gregory W. O'Neil,*[,a] John R. Williams,[a] Alexander M. Craig,[a] Robert K. Nelson,[b] Kelsey M. Gosselin,[b] and Christopher M. Reddy[b]

[a] *Department of Chemistry, Western Washington University, Bellingham, WA, 98225*

[b] *Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, MA, 02543*

* To whom correspondence should be addressed: Department of Chemistry, Western Washington University, 516 High Street, Bellingham, WA, 98225. Telephone: (360) 650-6283. Fax: (360) 650-2826. Email: oneilg@wwu.edu

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ABSTRACT. Polyunsaturated long-chain alkenones are a unique class of lipids biosynthesized in significant quantities (up to 20% of cell carbon) by several algae including the industrially grown marine microalgae *Isochrysis*. Alkenone structures are characterized by a long linear carbon-chain (35-40 carbons) with one to four *trans*-double bonds and terminating in a methyl or ethyl ketone. Alkenones were extracted and isolated from commercially obtained *Isochrysis* biomass and then subjected to cross-metathesis (CM) with methyl acrylate or acrylic acid using the Hoveyda-Grubbs metathesis initiator. Within 1 h at room temperature alkenones were consumed, however complete fragmentation (i.e. conversion to the smallest subunits by double bond cleavage) required up to 16 h. Analysis of the reaction mixture by gas chromatography and comprehensive two-dimensional gas chromatography revealed a predictable product mixture consisting primarily of long-chain (mostly C_{17}) acids (or methyl esters from CM with methyl acrylate) and diacids (or diesters), along

with smaller amounts (~5%) of the honey bee "queen substance" (*E*)-9-oxo-decenoic acid. Together, these compounds comprise a diverse mixture of valuable chemicals that includes surfactants, monomers, and an agriculturally relevant bee pheromone.

Introduction

As concerns about petroleum usage in connection with climate change persist [1], the need to transition toward renewable and sustainable raw materials is becoming increasingly relevant [2,3]. Others have commented that the replacement of petroleum in its many varied forms is, however, non-trivial [4]. It is prudent, therefore, to consider the range of alternative raw materials available to meet these demands. Based on a history of plant oil and sugar conversion processes, biomass-derived materials have remained at the forefront of many of these efforts [5, 6]. Plant oils generally refer to acylglycerols comprised of mainly C_{16} or C_{18} fatty acids (FAs) isolable from agricultural crops (e.g. soybean or rapeseed). Compared to terrestrial plants of this type, algae offer some advantages as a sustainable chemical feedstock. These include the ability to be grown using brackish, salt, or even wastewater thereby not competing for limited land and water resources [7]. Algae may also be sources of unique compounds representing new additions to our arsenal of sustainable chemicals [8]. One example is polyunsaturated long-chain alkenones [9], biosynthesized by a few species of haptophyte algae and with structures quite distinct from FAs. More specifically, alkenones contain hydrocarbon chains that are roughly twice the length of FAs (C_{35}-C_{40}) containing 1-4 *trans*-double bonds separated by five methylenes (as opposed to zero (i.e. saturated) or methylene-interrupted *cis*-alkenes common to FAs) and terminate in a methyl or ethyl ketone (Figure 1). Under certain conditions, alkenone content can exceed that of triglycerides, representing up to 20% of cell carbon [10, 11]. Neither the physiological role nor biosynthesis of alkenones is well understood, however it is proposed that they act for energy storage like other

lipids [12]. One such alkenone producer is *Isochrysis*, but others include the cosmopolitan coccolithophore *Emiliania huxleyi* and related *Gephyrocapsa oceanica* [13]. Here, we have focused on *Isochrysis* as it is one of only a few species of algae with a history of industrial cultivation, harvested for purposes of mariculture by several commercial suppliers worldwide [14]. Alkenones, therefore, represent a potentially abundant renewable carbon resource available from common algae with a history of large-scale production.

Recently, we demonstrated that alkenones can be efficiently separated and isolated from other lipids like FAs contained in the extract of commercial *Isochrysis* [15]. The approach generates multiple product streams, each representing a distinct platform from which to access different products. For instance, FAs can be converted to surfactants, esterified to biodiesel, or otherwise take advantage of the large volume of FA conversion technologies [16]. We argue that alkenones, with their unique structures and functionality, have the potential to provide access to a suite of compounds unobtainable from FAs. As an initial demonstration, we showed that alkenones can be converted to jet-fuel range hydrocarbons by means of cross-metathesis with 2-butene [17]. In this paper, we extend the metathesis repertoire of alkenones to include reactions with both methyl acrylate and acrylic acid (Scheme 1). The products from these reactions include long-chain esters (or acids), with hydrocarbon chains substantially longer than those obtained by the same reaction with common fatty acids (e.g. C_{17} from alkenones vs. C_{11} from oleic acid) [18]. These compounds would thus exhibit different (i.e. higher) hydrophilic-lipophilic balance values and thus novel properties as surfactants when compared to traditional fatty acid-based materials [19, 20]. Another product would be diesters, similar in structure to that obtained from acrylate cross-metathesis of fatty acids, however containing two α,β-unsaturated esters as sites for potential cross-linking [21]. Lastly, approximately 5% of (*E*)-9-oxo-2-decenoic acid (ODA), known as the "queen

substance" of the honey bee would be expected. This compound is a primary component of the queen mandibular pheromones [22-24], sold commercially as a honey bee attractant for agricultural purposes and representing a novel and valuable metathesis adduct from a renewable lipid feedstock [25].

Methods

Microalgae. The marine microalgae *Isochrysis* was purchased from Necton S.A (Olhão, Portugal) [14]. The algae were received as a dry milled powder that was yellow/brown in color.

Extraction and isolation of alkenones. Alkenones were isolated and purified from the *Isochrysis* biomass as previously described [26]. Briefly, Soxhlet extraction with hexanes produced a dark green near-black oily solid (mp. ~ 50-60 ºC, yield = 15% w/w) that we refer to as hexane algal oil [27]. This algal oil was then redissolved in methanol:dichloromethane (2:1, 10 x volume of algal oil) and treated with KOH (50% w/w) at 60 ºC for 3 h. The resulting saponified acylglycerols were selectively partitioned into water and the alkenone-containing neutral lipids extracted into hexanes. Removal of the hexanes gave the neutral lipids as a red-brown solid (approximately 50% w/w of the hexane algal oil) from which alkenones could be isolated by recrystallization with hexanes (45% w/w of the neutral lipids).

Alkenone acrylate cross metathesis, general procedure. To a mixture of alkenones (0.1 g; See Table 1) in dichloromethane (1.0 mL) was added methyl acrylate or acrylic acid (0.20 g, calculated as 6 equiv. relative to the most abundant alkenone (methyl 37:2, see Figure 1 and Table 1)) and Hoveyda-Grubbs catalyst (5 mg, 2-3 mol%) and the resulting mixture was stirred for the allotted time. Reactions were quenched with ethyl vinyl ether (0.9 mL, 50 equiv.) and stirred for 15 minutes before volatiles were removed on a rotary evaporator (See Supplementary Material). In some cases, methyl octadecanoate (0.1 g) was added as an inert internal standard to allow for

determination of percent conversions. For those reactions conducted with acrylic acid, the crude product mixture was first esterified (MeOH, cat. H_2SO_4) before analyzing by gas chromatography.

Analysis by ^{1}H nuclear magnetic resonance (1H NMR) spectroscopy. ^{1}H NMR spectra of the purified alkenones and cross-metathesis reaction mixtures were obtained under ambient conditions using $CDCl_3$ as solvent, which also served as internal reference (shift value of residual proton at 7.26 ppm).

Analysis by one-dimensional gas chromatography with flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS). The purified alkenones and cross-metathesis reactions were analyzed on a Hewlett-Packard 5890 Series II GC-FID. Samples (1 μl) were injected cool-on-column and separated on a 100% dimethyl polysiloxane capillary column (Restek Rtx-IMS, 30 m length, 0.25 mm I.D., 0.25 μm film thickness) with H_2 as the carrier gas at a constant flow of 5 mL min^{-1}. The GC oven was programmed from 70 ℃ (7 min hold) and ramped at 6 ℃ min^{-1} to 320 ℃ (15 min hold). Percent conversions for the cross-metathesis reactions were determined by comparison of integration ratios for combined alkenones (rt = 44-48 min) to methyl stearate (retention time = 27.5 min) relative to a starting alkenone/methyl octadecanoate standard mixture. Select samples were also analyzed by GC-MS (full scan) on an Agilent 6890 GC with a 5973 MSD. Splitless 1 μL sample injections, were separated on a DB-XLB capillary column (60 m x 0.25 mm x 0.25 μm film thickness) using helium as the carrier gas (10.5 psi constant pressure), and the following GC temperature program: 4 min at 40 °C and ramped to 320 at 5 °C min^{-1} (held 15 min).

Analysis by comprehensive two-dimensional gas chromatography with flame ionization detection (GC×GC-FID) and time of flight mass spectrometer (GC×GC-TOF). Select cross-

metathesis reaction mixtures were analyzed by GC×GC−FID and GC×GC-TOF MS according to previously described methodologies (see Supplementary Material).

Results and Discussion

Isolation and analysis of purified alkenones. The procedure employed for extracting and purifying alkenones from *Isochrysis* biomass was the same that has been previously described involving Soxhlet extraction with hexanes followed by saponification and separation [15, 24]. Overall yields of lipids (i.e. "hexane algal oil") were generally 15% w/w of the dry biomass. This is similar to the value stated by the manufacturer Necton, who describes their product as containing 40% protein and 20% fat. Analysis of the purified alkenones isolated from this lipid mixture by gas chromatography revealed the presence of primarily methyl C37:3, methyl C37:2, and ethyl C38:2 alkenones (where methyl and ethyl refer to methyl or ethyl ketones and C#:# is the number of carbon atoms:number of double bonds, *ref.* Figure 1) with the most abundant being the methyl 37:2 (Table 1).

Alkenone unsaturation is influenced by growing temperature such that at colder temperatures alkenones become more highly polyunsaturated [29]. This is an important (and pontentially strategic) consideration for metathesis-based conversion technologies as the starting alkenone mixture directly impacts the identity and yields of products obtained. From the data in Table 1, we can calculate the growing temperature (T) for the *Isochrysis* used in this study from the so-called unsaturation index ($U^{K'}_{37}$, eq. 1, where $C_{37:2}$ and $C_{37:3}$ refers to the relative amounts of these alkenones) [30-35]:

$$U^{K'}_{37} = C_{37:2} / (C_{37:2} + C_{37:3})$$
$$U^{K'}_{37} = 0.033T\,[^{\circ}C] + 0.044 \quad (1)$$

Using our ratio of $C_{37:2}$ to $C_{37:3}$ alkenones (2.9:1) and associated $U^{K'}_{37}$ value (0.744), we calculate that the *Isochrysis* was grown at 21.2 ºC, corresponding to average temperatures in April and October/November for our supplier's region (Olhão, Portugal) [36]. For comparison, algae grown during the coldest month (January, Avg. = 16 ºC) would have given an alkenone mixture with a significantly different 1.34:1 ($U^{K'}_{37}$ = 0.572) $C_{37:2}$ to $C_{37:3}$ ratio. While this sensitivity to growing temperature presents an opportunity to enrich or select for one product type by metathesis (e.g. diacids from algae grown at colder temperature with more polyunsaturated alkenones), it becomes critical that extracted alkenones be accurately characterized before planning for certain product compositions even when working with biomass procured from a single supplier.

Alkenone/acrylate cross metathesis. Treatment of alkenones with the Hoveyda-Grubbs' catalyst in the presence of an excess of methyl acrylate at room temperature for 16 h led to complete consumption as confirmed by both ^{1}H NMR and GC, with mass recoveries consistently quantitative (Scheme 2). From the profile presented in Table 1, we could predict what were to be the major products from this reaction. For instance, cross-metathesis with methyl acrylate was expected to produce the diester undeca-2,9-dienedioate (**1**) as the major single compound (37.8%, Table 2) representing a suitable monomer with which to access polymers like polyesters [37] and polyamides [21]. Approximately 7% was predicted be the methyl ester of the so-called "queen substance" of the honey bee, methyl 9-oxodec-2-enoate (methyl ODA, **2**), derived from the methyl 37:3 alkenone and of value for agricultural purposes [25]. The remainder (46%) would be comprised of C_{16} (compounds **4** and **5**) and C_{17} (compound **3**) esters. Combined with **1**, roughly 95% of the products would target high-volume applications such as polymers (i.e. **1**) and surfactants (i.e. **3-5**)

As illustrated by the GC-FID chromatogram in Figure 2, the actual product mixture from this reaction contained more compounds than those included in Table 2. Identification of these minor components is critical to a complete understanding of this cross-metathesis process. For this reason, we chose to employ comprehensive two-dimensional gas chromatography (GC×GC) for the analysis of our alkenone-acrylate cross-metathesis product mixtures.

GC×GC has a history of success in analyzing exceedingly complex mixtures of compounds, particularly for petroleum research [38], but more recently applied to reaction development [39]. The technique uses two serially joined columns whereby all compounds eluting from the first are trapped and then reinjected onto the second. The result is better resolution and enhanced signal to noise plus, as shown in Figure 3, different chemical classes align into groups (or fairways) based on their retention in the second dimension within a GC×GC chromatogram making for enhanced interpretation. For this particular experiment, the three major product subclasses: dimethyl esters, long-chain ketoesters, and monomethyl esters (*ref.* Scheme 2) are readily distinguishable. Our instrument was configured with a polar second-dimension GC column such that the least polar monomethyl esters (e.g. methyl heptadec-2-enoate (**3**)) eluted first followed by intermediate polarity ketoesters (e.g. methyl 9-oxoheptadec-2-enoate (**4**)) and the most polar diesters eluted last.

Coupling of GC×GC with a flame ionization detector (FID) allows for the necessary separations but also the capacity to get accurate concentrations of every compound within the GC×GC chromatogram because most hydrocarbons have similar response factors [40]. Quantifying the results from analysis of the product composition by GC×GC-FID revealed some differences with those predicted (*ref.* Table 2). For instance, the amount of diester **1** was diminished (27.5% vs 37.8% predicted). Cross-metathesis with acrylic acid gave a nearly identical

product composition (after esterification) under the same conditions suggesting that the nature of the cross-metathesis partner was not responsible for the discrepancy (e.g. 27.2% of **1**, see Table 2). Combined, compounds **1**-**5** represented 77% of the total mixture. Compound **1** was obtained as a 90:8:2 mixture of *E*,*E*:*E*,*Z*:*Z*,*Z*-isomers (Figure 3, 1st domention retention time = 66 – 70 min.; 2nd dimension retention time = 2.6 – 3.6 min.). It is interesting to compare the selectivities obtained from this cross-metathesis with methyl acrylate to those we reported for the similar reaction with 2-butene ("butenolysis") [17]. Overall, the methyl acrylate/alkenone cross-metathesis was more *trans*-selective, giving ≥ 90% of the thermodynamically favored *trans*- (or *trans,trans*) products (vs. 80% from the butenolysis). Irrespective of the geometric isomer composition, all isomers of **1** and both *E* and *Z* compounds **3**-**5** would be expected to provide similar entry to cross-linked polymers and surfactants respectively [41].

Similar to cross-metathesis with 2-butene [17], in addition to the C_{17} methyl ester **3**, we observed a small amount of an unexpected homologous series of long-chain esters from the reaction with methyl acrylate (e.g. C_{16}-C_{19}, Figure 3). Previously we attributed differences between predicted identities and amounts of products and those obtained to double bond isomerization that can occur during metathesis [42]. Jenkins et al. also recently reported small amounts of isomerization (from terminal to internal alkenes) during their ethenolysis of microalgal triglycerides [43]. As double bond placement likely has a minimal impact on fuel properties, the authors argued that isomerization is not problematic for fuels. However, as an indicator for catalyst decomposition isomerization should be avoided.

Metathesis isomerization is thought to arise from the formation of a ruthenium-hydride [42], which would also explain our observation of small amounts of what we have tentatively assigned as reduced cross-metathesis products dimethyl undec-2-enedioate and methyl 16-

oxooctadecanoate (Figure 3, peaks (a) and (b) respectively) [44]. Certain additives have been shown to inhibit these processes, presumably by interaction with the unwanted metal-hydride species. To that end, both phenol and benzoquinone were tested as isomerization inhibitors and the reaction mixtures analyzed by GC×GC-FID [45, 46]. As indicated in Table 3, the use of these additives had little effect on product ratios. It may be that these compounds are ineffective in inhibiting isomerization for this particular reaction, or that *Isochrysis* biosynthesizes small amounts of alkenones with differing double bond positions that would then be relayed into the observed metathesis product distribution.

In order to investigate the kinetics of the reaction, we monitored alkenone conversion throughout the reaction. When attempting a similar analysis of alkenone cross-metathesis with 2-butene, we encountered difficulties due to the low temperatures required to condense 2-butene which caused insolubility for the alkenones [17]. Cross-metathesis reactions with methyl acrylate or acrylic acid, however, were conducted at room temperature (boiling points = 80 and 139 ºC respectively) thus maintaining alkenone solubility and allowing for kinetic analysis by sampling (and quenching) a single reaction at various time increments [47]. Results from this experiment are presented in Table 4 and the accompanying gas chromatograms in Figure 4.

As shown, the reaction gave 99% conversion after 30 min which is a similar timeframe observed for the previous 2-butene/alkenone cross-metathesis (99.5% after 30 min) [17], despite the significant electronic differences between 2-butene and methyl acrylate which can greatly influence olefin metathesis reactivity [48]. The rate of conversion for our alkenone metathesis is also identical to that reported for the same reaction with methyl oleate under similar conditions [18], suggesting that lipid alkene geometry (*trans* for alkenones vs. *cis* for methyl oleate) is not limiting in this respect.

Additional results can be obtained beyond those in Table 4. More specifically, monitoring alkenone loss does not allow for differentiation between complete (i.e. reaction at all of the double bonds within a molecule) vs. incomplete metathesis (e.g. reaction at only one double bond). While this information could be obtained by GC×GC analysis [49], for this particular reaction the use of ^{1}H NMR proved convenient. Previously we noted that NMR was not helpful when analyzing alkenone cross-metathesis reactions with 2-butene because the spectra for the starting alkenones and resulting products were too similar [17]. Cross-metathesis reactions with methyl acrylate, however, gives products with alkene proton signals quite distinct from the starting alkenone double bond protons (*ref.* Scheme 1). By tracking formation of these new alkene signals (δ 6.96 and 5.81 ppm) from the cross-metathesis products and loss of intact alkenone double bonds (δ ~5.4 ppm) in their ^{1}H NMR spectra, we could then determine at what point complete fragmentation had occurred. Combined with the GC data (Figure 4), we conclude that while alkenones are consumed within 1 h, complete metathesis under these reaction conditions (~6 equiv. methyl acrylate, 5 mol% catalyst, room temperature) takes upwards of 16 h (Figure 5).

Conclusion

Alkenones are a unique class of lipids biosynthesized by certain species of algae including *Isochrysis* that are currently grown industrially and available for purchase in multi-kilogram quantities from several suppliers internationally (e.g. USA and Portugal [14]). Structurally, alkenones are quite distinct from the fatty acids that have historically served as the basis for various renewable chemical enterprises, thereby providing a means to access novel products as part of ongoing petroleum replacement efforts. In this study, alkenones that had been extracted and isolated from commercial *Isochrysis* biomass were engaged in cross-metathesis reactions with methyl acrylate and acrylic acid. Using the Hoveyda-Grubbs metathesis initiator, alkenones were

completely consumed within 1 h at room temperature, however complete metathesis (i.e. conversion to the smallest subunits) required longer reaction times (approximately 16 h). Product mixtures were analyzed by GC×GC, which revealed slightly different ratios of products than what was calculated based on the starting alkenone profile. In particular, the amount of dimethyl undeca-2,9-dienedioate (**1**) obtained was 10% less than what was expected. It was hypothesized that isomerization during the metathesis reaction contributed to this difference, however the use of additives reported to suppress isomerization during these reactions had little effect on the product distribution. Nonetheless, all of the predicted major products including long-chain (mainly C_{17}) esters and bis-α,β-unsaturated diesters along with small amounts of the queen honey bee pheromone were obtained, reflective of an overall well-behaved renewable feedstock to fine chemical conversion process. The work provides a demonstration of the novel products that can be afforded by alkenones owing to their unique structures. Moreover, because alkenone structure can be manipulated using well-established (e.g. growing temperature [29-35]) or other emerging technologies (e.g. bacterial interactions [50]), a next phase of research may include the use of "designer" *Isochrysis* with optimized alkenone contents targeted toward specific product classes.

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Supplementary Material. GCxGC data.

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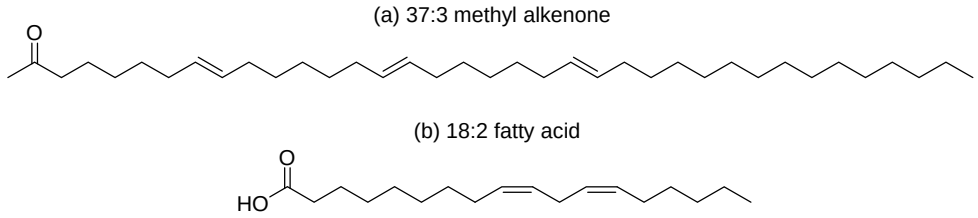

Figure 1. Structures of a common alkenone produced by *Isochrysis* sp. (a, where methyl refers to a methyl ketone) and common fatty acid linoleic acid (b). Nomenclature for both is # of carbons:# of double bonds, however note that the configuration of double bonds is different.

Scheme 1. Comparison of fatty acid methyl oleate and alkenone-acrylate cross-metathesis.

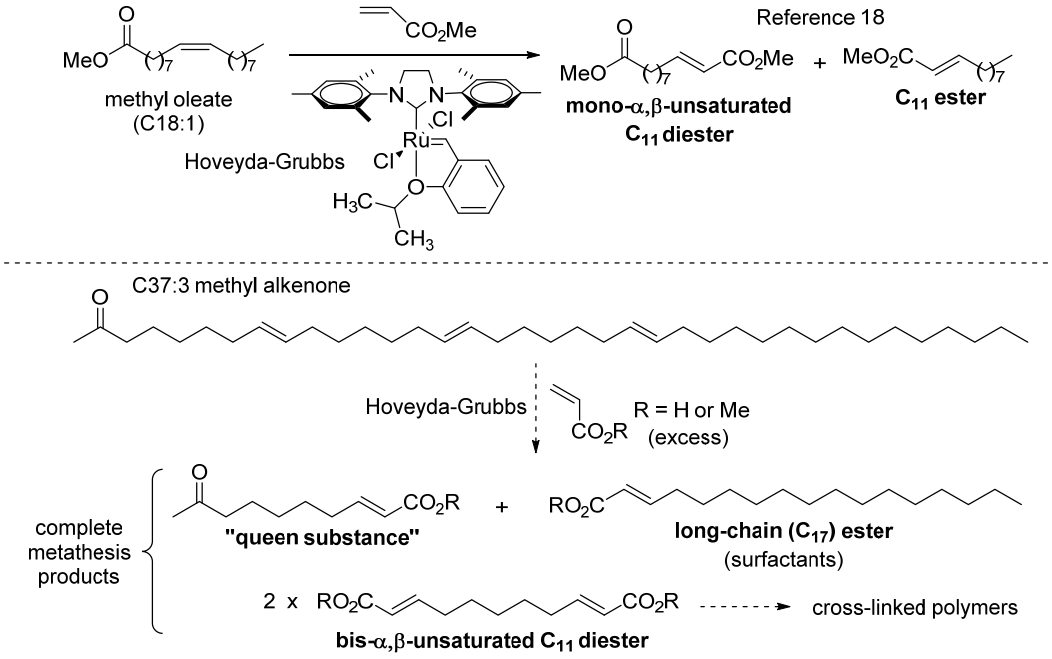

Table 1. Isolated *Isochrysis* alkenone composition.

Alkenone (*trans*-double bond position)[A]	Relative %[B]
methyl 37:3 (8,15,22)	21.6
methyl 37:2 (15,22)	62.7
ethyl 38:2 (16,23)	15.7

Notes for Table 1: [A]Determined by GCMS. [B]Determined by GC-FID.

Scheme 2. Cross-metathesis of major isolated *Isochrysis* alkenones with methyl acrylate.

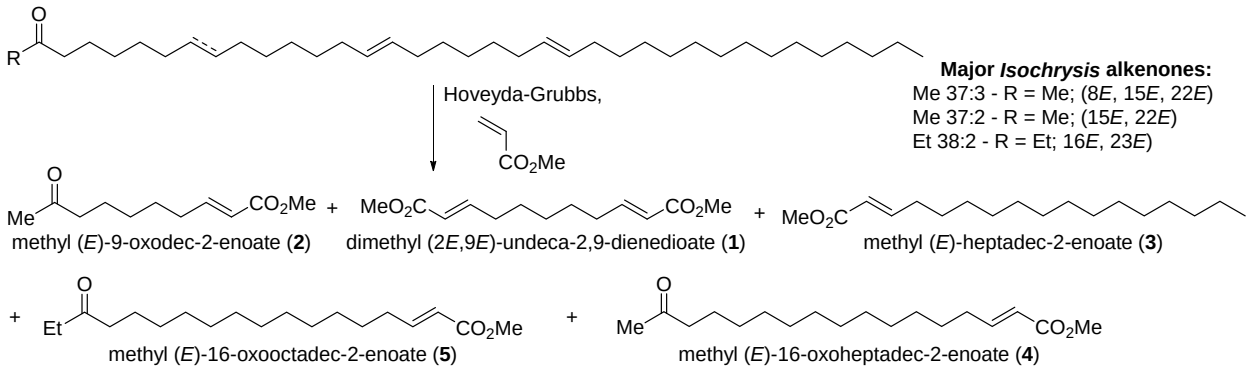

Table 2. Predicted and measured relative amounts of products from the cross-metathesis of alkenones with methyl acrylate and acrylic acid (after esterification) catalyzed by the Hoveyda-Grubbs catalyst.

Compound[A]	**Predicted Relative %[B]**	**Measured Relative % (reaction with acrylic acid)[C]**
dimethyl undeca-2,9-dienedioate (**1**)	37.8	27.5 (27.2)
methyl heptadec-2-enoate (**3**)	31.1	41.9 (43.2)
methyl 16-oxoheptadec-2-enoate (**4**)	19.5	18.3 (17.0)
methyl 9-oxodec-2-enoate (**2**)	6.7	5.5 (6.3)
methyl 16-oxooctadec-2-enoate (**5**)	4.9	6.8 (6.3)

Notes for Table: [A]Sum of geometric isomers. [B]Calculated by: Total moles of compound **1** = $2X + Y + Z$; **2** = X; **3** = $X + Y + Z$; **4** = Y; and **5** = Z (where X = relative moles of 37:3 methyl alkenone, Y = moles of 37:2 methyl alkenone; and Z = moles of 38:2 ethyl alkenone). [C]Determined by GC×GC. Combined, compounds **1**-**5** represent 77% of the total mixture (see Supplementary Material and Figure 3)

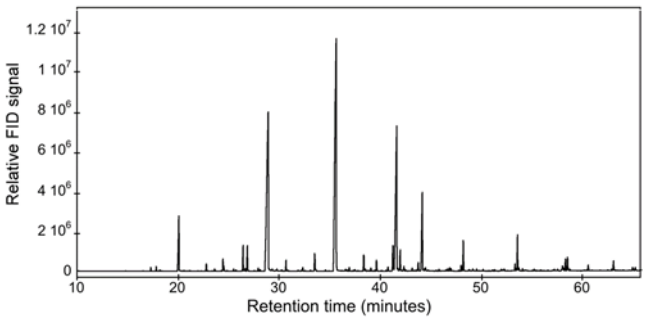

Figure 2. GC-FID chromatogram of alkenone/methyl acrylate cross-metathesis reaction mixture showing many minor and potentially co-eluting products in addition to the five compounds indicated in Table 2.

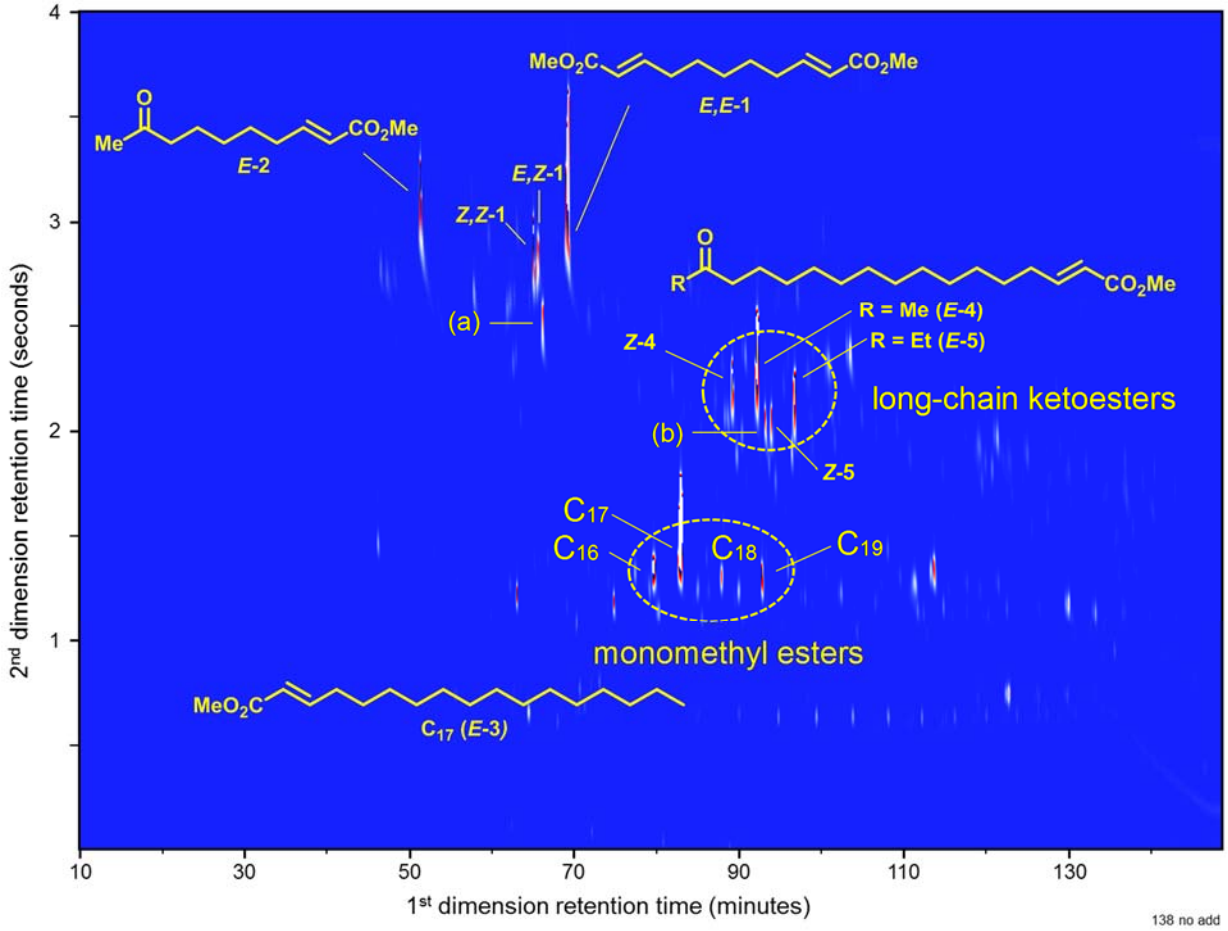

Figure 3. GC×GC-FID chromatogram (plan view) of the alkenone-methyl acrylate cross-metathesis product mixture showing resolution of *E*:*Z* isomers and separation of compounds into different subclasses including a homologous series of long-chain (e.g. C_{16} – C_{19}) α,β-unsaturated esters. First dimension retention times are based on vapor pressures whereas the second dimension is polarizability. The peaks labeled (a) and (b) have been tentatively assigned to reduced cross-

metathesis products dimethyl undec-2-enedioate (*m/z* = 242) and methyl 16-oxooctadecanoate respectively (*m/z* = 312) when analyzed by GCxGC-MS. Combined, the labeled compounds represent 84% of the total products (see Supplementary Material).

Table 3. Relative amounts of products obtained by cross-metathesis of alkenones and methyl acrylate with or without isomerization inhibitors phenol and benzoquinone.

Compound	**No Additive**	**Phenol[A]**	**Benzoquinone[B]**
dimethyl undeca-2,9-dienedioate	27.5	27.2	27.2
methyl heptadec-2-enoate	41.9	43.5	42.6
methyl 16-oxoheptadec-2-enoate	18.3	18.1	18.7
methyl 9-oxodec-2-enoate	5.5	4.6	4.6
methyl 16-oxooctadec-2-enoate	6.8	6.6	6.9

Notes for Table: [A]0.5 equiv. [B]0.1 equiv.

Table 4. Kinetic analysis of alkenone cross-metathesis with methyl acrylate.

Time (min.)[A]	**% Conversion[B]**
15	85
30	99
60	99.9

Footnote for Table 4: [A]Aliquots were removed at the times indicated and then quenched with ethyl vinyl ether (50 equiv.) before concentrating *in vacuo* and analyzing be GC. [B]Percent conversions were determined by comparing the integration ratios for combined alkenones to methyl stearate (inert internal standard) relative to a starting alkenone/methyl stearate reference mixture (See Figure 4).

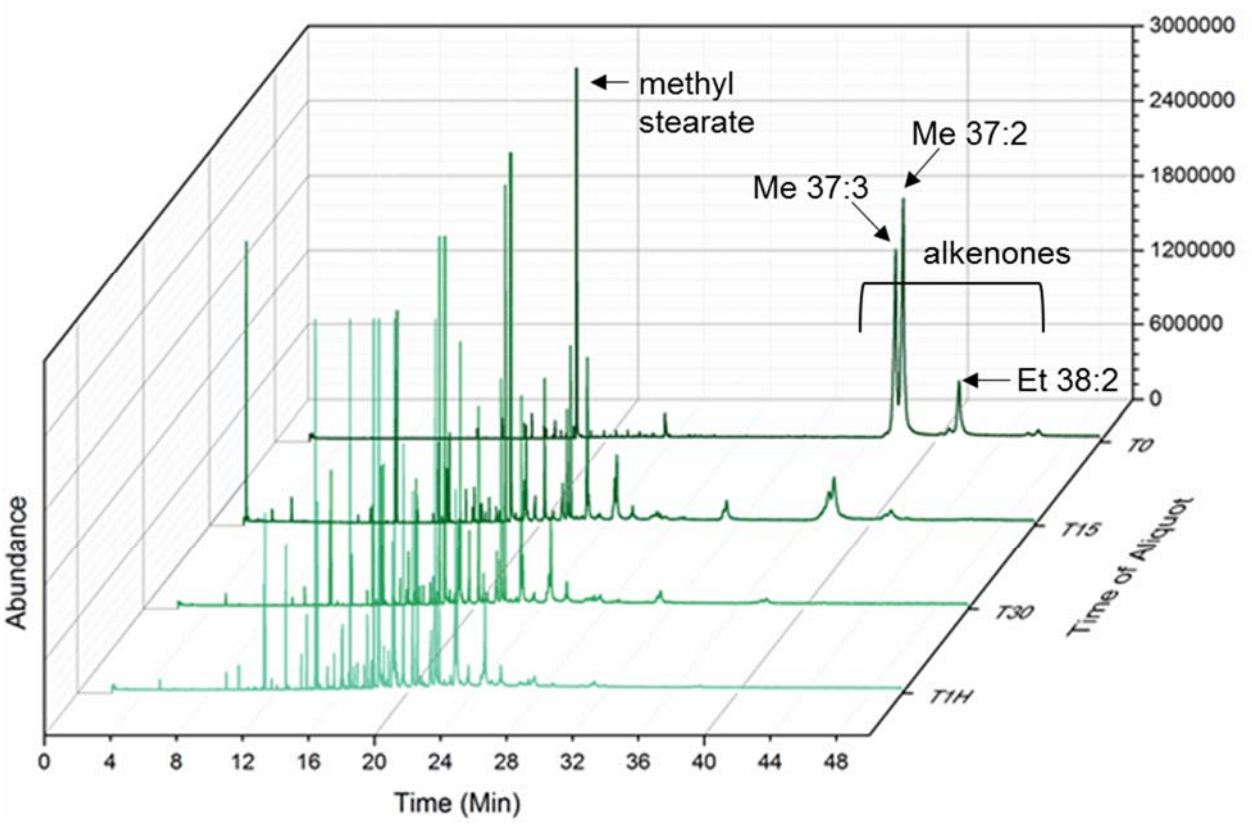

Figure 4. GC-FID chromatograms of aliquots taken from the cross-metathesis of alkenones with methyl acrylate and quenched (ethyl vinyl ether) at the times indicated showing essentially complete conversion within 1 hour (T1H).

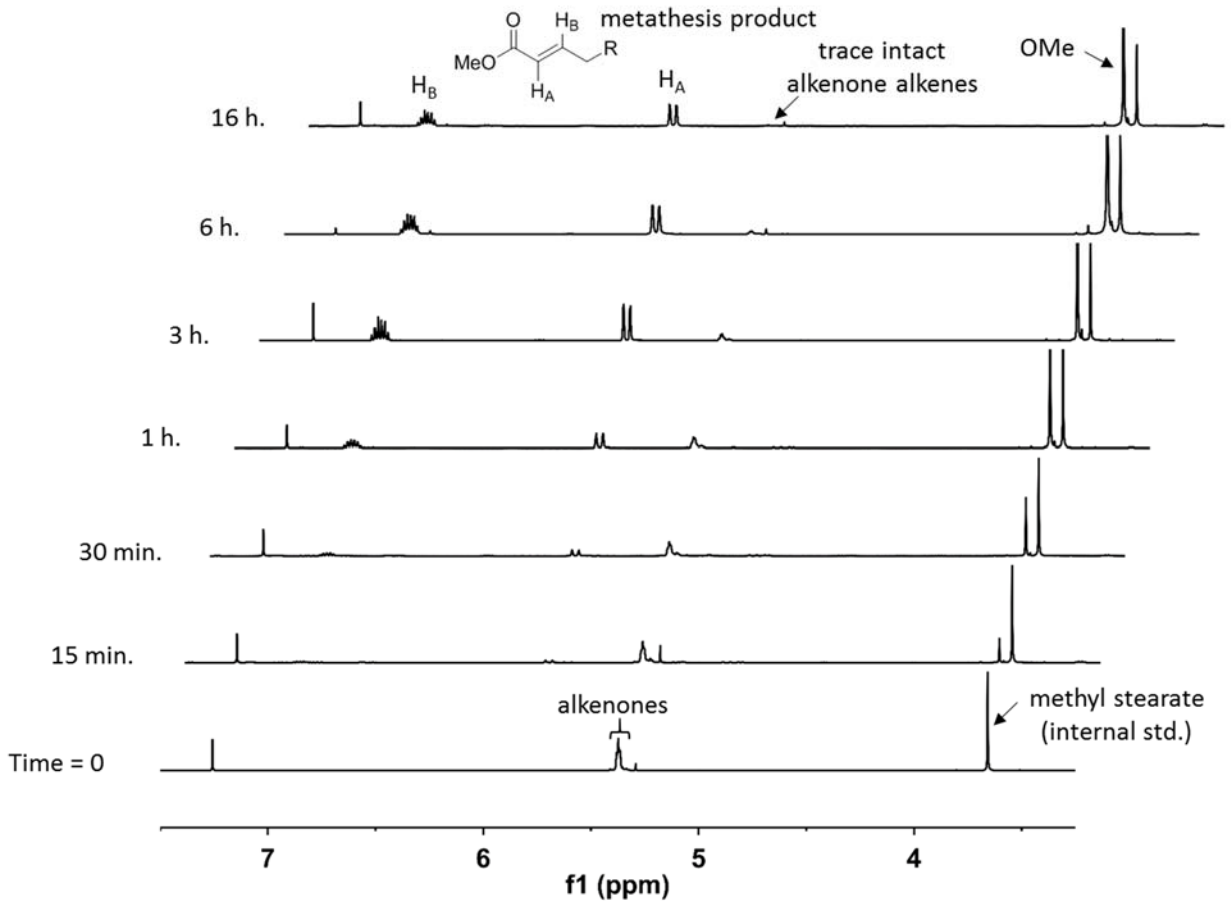

Figure 5. ^{1}H NMR spectra of aliquots from the cross-metathesis reaction of alkenones with methyl acrylate quenched at the times indicated. Signals corresponding to products and intact alkenones were sufficiently different that this analysis allowed for determination of the time required for complete metathesis (i.e reaction at all of the alkenone double bonds, *ref.* Scheme 2).