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Structure Elucidation

Simple Determination of Double-Bond Positions in Long-Chain Olefins by Cross-Metathesis**

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The accurate determination of the double-bond positions in unsaturated long-chain compounds remains a challenging task, even in this advanced spectroscopic era.[1] Although NMR spectroscopy is the principal method of structural determination, it does not provide adequate information regarding the double-bond positions in unsaturated chains, owing to highly overlapped signals in NMR spectra. Thus, current analytical approaches for this purpose rely on mass spectrometry (MS).[2,3]

Direct analysis of double-bond positions in the analyte with a conventional form of MS, such as electron impact (EI) or chemical ionization (CI) MS, is inherently unreliable.[1,4] This unreliability occurs because EI ionization causes rapid isomerization of the molecular ions, and CI with conventional proton-transfer reagents often fails to yield useful fragments. These problems could be solved either by using advanced mass spectrometric equipment or special CI reactant gases[2,5] or both. However, these methods have limited potential for non-specialist laboratories, and they are not adapted to routine experiments.

More detailed and specific mass information on the double-bond position can be acquired from derivatives of the target analyte. Various types of chemical reactions have been employed for derivatization.[6] Some representative reactions are ozonolysis,[7] dihydroxylation,[8] alkylthiolation,[9] epoxidation,[10] and methoxymercuration.[11] One drawback of these past chemical derivatization approaches is that the above reactions do not generally provide compounds with physicochemical properties adequate for chromatographic mass spectrometry. Thus, to acquire the desired properties of the analyte, a second derivatization is necessary in most cases. An additional drawback is the possible interference of other functional groups with the analyte during the derivatizations, which could cause failure in forming the expected derivatives and thus lead to ambiguous mass information. Consequently, there still remains a need for simple and reliable methods to identify the double-bond positions with high accuracy and capacity.

Olefin cross-metathesis (CM) is a metal-catalyzed process that yields a new carbon–carbon double bond by an intermolecular mutual exchange of alkylidene fragments between two olefins.[12] This versatile reaction has had a wide and profound impact in diverse areas of chemistry. However, the application of CM in the field of analytical chemistry is rare. To the best of our knowledge, there is only one report concerning an application of CM for analytical purposes. Gee and Prampin used CM to estimate the positions of double bonds in mixtures of linear olefins.[13] Their GC/MS-based method requires about a gram of sample and a specialized computer algorithm, and it has some limitations because it is only applicable to a mixture of unsaturated hydrocarbons with one double bond.

We envisioned that a CM reaction of unsaturated long chains with a simple olefin could afford derivatives suitable for chromatographic mass spectrometry without the need for an additional derivatization step. The exhaustive interpretation of the fragment ion mass spectra of CM products is not required to determine the original double-bond position. Instead, the position can be simply deduced from the values obtained by comparing the mass changes between the starting materials and the CM products and considering the structural characteristics of the starting olefins. Compared to conventional oxidative derivatization, the reaction conditions of CM are mild enough to tolerate a variety of functional groups. Thus, CM-based position determination could be applied to complicated compounds. Herein, we present our studies on the development of a simple and reliable CM-based method for the determination of the double-bond position in long chain compounds by either LC/MS or GC/MS.

For a successful application of the CM reaction to the analysis of double-bond positions, the CM partner and CM catalyst should fulfill the following criteria: 1) CM partner and catalyst should be easily available; 2) they can be handled on the bench without any special equipments or techniques; 3) they should maximize the formation of CM products, without giving a mixture of geometrical isomers, for clear interpretation of data; and 4) they must not impede mass spectrometry analysis of the products or can be easily removed.

Based on the criteria listed above, we surveyed several metathesis catalysts and CM partners to discover an optimal set of reaction components. This effort resulted in the selection of two sets of reaction components (Scheme 1): set A is methyl acrylate (**1**)/second-generation Hoveyda–

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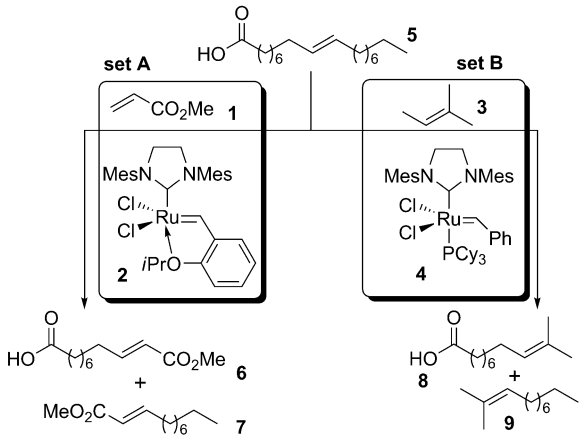

Scheme 1. The olefin cross-metathesis of **5** with set A and set B.

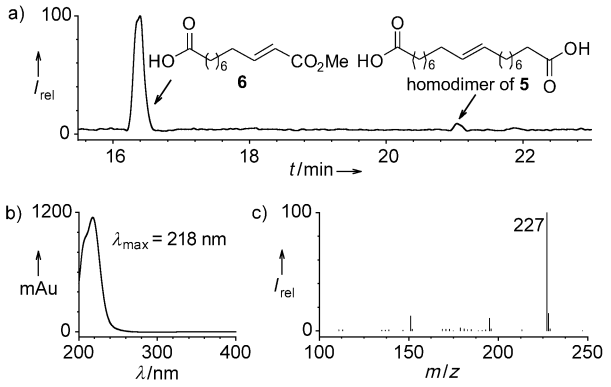

Figure 1. LC/MS analysis of the CM products from elaidic acid **5**. a) Liquid chromatogram; b) the UV spectrum of the peak at 16.4 min in the LC; and c) the ESI negative ionization mode mass spectrum of the peak at 16.4 min in LC.

Grubbs catalyst **2**, and set B is 2-methyl-2-butene (**3**)/ second-generation Grubbs catalyst **4**.[14] The CM reaction of elaidic acid (**5**) with set A in CH_2Cl_2 led to the formation of CM products **6** and **7** with the *E* configuration in high yields, and the set B formed *gem*-dimethyl olefins **8** and **9**.

We envisioned that set A would be useful for developing an LC/MS-based analytical method because set A generates a CM product with increased hydrophilicity that is preferable for reverse-phase LC. Furthermore, the UV-active α,β-unsaturated ester functionality makes it possible to unambiguously identify the CM product by LC/MS with a common diode-array UV detector, even with interference from non-specific signals. However, it was expected that set B would be suitable for GC/MS analysis because set B generates, at least in part, a CM product that is more volatile relative to the starting substrate.

After finding two sets of reaction components, we explored the optimized reaction conditions and procedures that are suitable for chromatography–mass spectrometry. After extensive efforts, we could set up the standard procedure A (PA) for LC/MS analysis:[15] 1) Dissolve a target molecule (≤0.5 mg) in CH_2Cl_2/methyl acrylate 10:1 mixture (0.5 mL); 2) add 50 μg (5–10 mol%) of second-generation Hoveyda–Grubbs catalyst **2**; 3) stir at room temperature for 2–3 h; 4) inject 10 μL of the reaction mixture into an LC/MS (conventional C_{18} column, H_2O/MeOH 0.1% formic acid gradient solvent) without any treatment; and 5) analyze the data based on the mass values.

The LC/MS spectrum of the analyte, obtained from elaidic acid (**5**) using procedure PA, is shown in Figure 1. The major peak showed UV absorbance at 218 nm, which is typical of the α,β-unsaturated ester (Figure 1b). Its identity could be deduced by the molecular ions: $[M-H]^-$ at *m*/*z* 227 in the ESI negative ionization mode and $[M+H]^+$ at *m*/*z* 229 in the positive mode (Figure 1c and Supporting Information). The obtained molecular mass (228 Daltons) of this CM product matched well with the theoretical value for **6**, and this result clearly indicated that the double-bond position is at C-9 of elaidic acid. The homodimerized product was also detected as a minor peak (Figure 1a). However, only low levels of the other CM product **7** were observed, possibly as a result of inefficient ionization.

To validate procedure PA, we applied it to various unsaturated fatty acids. As shown in Table 1, all of the tested fatty acids successfully yielded the expected CM products, regardless of their geometry or position of the double bond. The fatty acid positional isomers gave CM products with mass values that differed from each other (entries 1–4, Table 1). Octadecenoic acid and erucic acid produced the same CM fragment (entries 4 and 6) because their double bond occurs at the same number of carbon atoms away from the carboxylic group. However, this similarity is not a hurdle in assigning double-bond positions because these two fatty acids can be easily distinguished by molecular mass.

Table 1: Cross-metathesis-based LC/MS analysis of various fatty acids.

Entry	Fatty acids	MW	MW of CM product[a]
1	petroselinic acid (18:1, 6*Z*)	282	186
2	oleic acid (18:1, 9*Z*)	282	228
3	*cis*-vaccenic acid (18:1, 11*Z*)	282	256
4	*trans*-13-octadecenoic acid (18:1, 13*E*)	282	284
5	*cis*-11-eicosenoic acid (20:1, 11*Z*)	310	256
6	erucic acid (22:1, 13*E*)	338	284
7	nervonic acid (24:1, 15*Z*)	366	312
8	linoleic acid (18:2, 9*Z*,12*Z*)	280	228
9	arachidonic acid (24:4, 5*Z*,8*Z*,11*Z*,14*Z*)	304	172

[a] Standard procedure PA was employed.

A long-chain fatty acid with two double bonds, namely linoleic acid, led to the detection of the CM product that arose from the first double bond from the carboxylic terminus

(entry 8). The peak corresponding to the product derived from the reaction at the second double bond was not detected.[16] A similar pattern was also observed for arachidonic acid, which has four double bonds (entry 9). These results indicate that set A results in the predominant formation of the CM product of the first double bond, regardless of the number of double bonds in long linear olefins.

As mentioned above, CM set B would be useful for GC/MS analysis because the CM products using 2-methyl-2-butene (**3**) would be more volatile than those produced using methyl acrylate (**1**). Furthermore, **3** can easily be removed from the final products owing to its low boiling point (39 °C). Therefore, the standard GC/MS procedure PB was developed through an optimization process. One notable difference between PA and PB is that extra manipulation is necessary to remove the nonvolatile ruthenium byproducts that are generated from the catalyst with activated carbon for the protection of the capillary column.[17]

The suggested procedure PB is as follows:[15] 1) Dissolve a target molecule ($\leq$0.5 mg) in CH_2Cl_2/2-methyl-2-butene 1:1 mixture (0.5 mL); 2) add 50 μg (5–10 mol%) of the second-generation Grubbs catalyst; 3) stir at room temperature for 2–3 h; 4) add 50 mg of activated carbon and stir for 10 min; 5) filter the reaction mixture with the eluent (CH_2Cl_2/Et_2O 5:1) through a pipette column filled with 300 mg of silica gel; 6) remove 2-methyl-2-butene and eluent at low vacuum (approximately 60 mbar) on a rotary evaporator; 7) dissolve the residue in 2 mL of CH_2Cl_2; 8) inject the dissolved material into a GC/MS with a conventional silica column; and 9) analyze the data based on the mass values.

Figure 2 shows the GC/MS profiles of the CM products that were obtained from elaidic acid (**5**) and its methyl ester derivative using PB. The gas chromatogram of the analyte

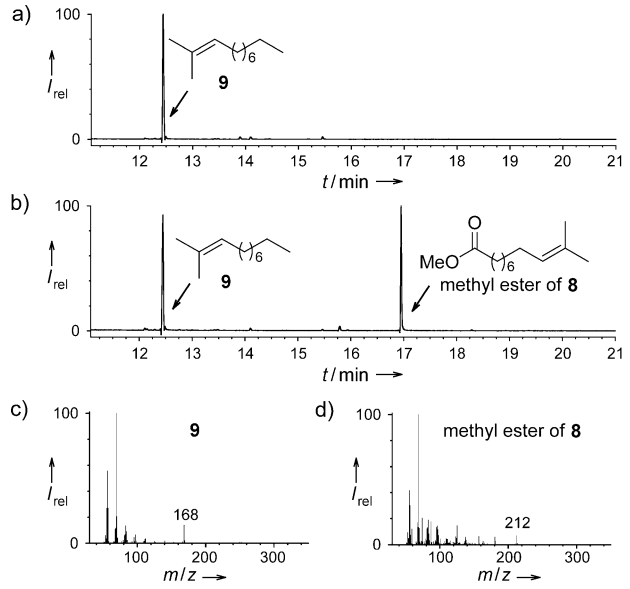

Figure 2. GC/MS analysis of the CM products from elaidic acid **5** and its methyl ester. Gas chromatograms of a) elaidic acid and b) elaidic acid methyl ester; mass spectra (EI 70 eV) of the peaks at c) 12.5 min and d) 17.0 min in GC.

obtained from fatty acid **5** showed only one predominant peak corresponding to the CM product **9**.[18] The peak corresponding to the other possible product **8**, containing a carboxylic acid group, was not observed during the monitored period owing to its high polarity and boiling point. However, the chromatogram derived from the methyl ester of **5** exhibited two peaks of similar intensity (Figure 2b). A similar pattern was also observed for other fatty acids and their ester derivatives, including nervonic acid (see Supporting Information).

It is notable that our CM-based GC/MS analytic approach to unsaturated fatty acids, unlike most other oxidative derivatization approaches, does not require prior esterification of the acid group because the CM reaction of fatty acid with **3** certainly affords a nonpolar product suitable for GC.

To further demonstrate the utility of our positioning method, we applied the standard CM-based LC/MS procedure (PA) to the natural product olvanil, which has a molecular weight of 417 (Figure 3). In this experiment, we

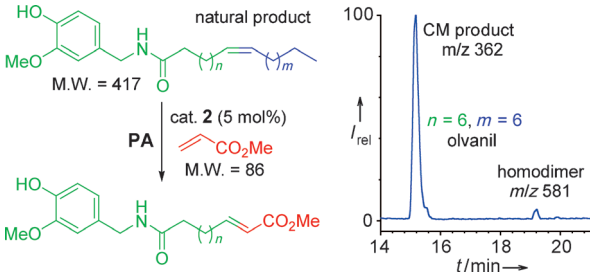

Figure 3. Determination of the position of the double bond in olvanil by PA.

pretended not to know the exact position of the double bond. The LC/MS chromatogram of the analyte had two peaks: a major peak of the molecular ion at m/z 362 ($[M-H]^-$) and a minor peak at 581. Because the mass value of the minor peak is larger than the sum of the individual mass values of olvanil and methyl acrylate. The minor peak is assigned as a homodimerization product of olvanil. The double-bond position could easily be deduced from the value obtained from the major peak.[19] The sum of the mass values of olvanil and methyl acrylate is 503, and the molecular mass of the product is 363. The difference between the two values is 140, which corresponds to the mass of $C_{10}H_{20}$. Because acrylate inherently contributes one carbon atom to the deleted ten carbon atoms, nine carbon atoms out of ten originate from olvanil. Consequently, it can be deduced that olvanil has a double bond at the ninth carbon position from the methyl terminus.

In conclusion, we developed a new chemical derivatization method for the determination of double-bond positions in unsaturated long-chain compounds. The method is based on the cross-metathesis reaction between a target compound and a simple olefin, which results in the formation of fragmented olefinic products. Depending on the cross-metathesis partner used, the produced CM fragments have distinct physicochemical properties that are suitable for LC/MS and

GC/MS analyses. The position of the double bond can easily be deduced by comparing and analyzing the molecular mass changes. Both of the presented LC/MS and GC/MS approaches are equally reliable, and either one can be chosen depending upon the availability of the instruments. Compared to conventional chemical derivatization approaches, our proposed CM-based approach could be applicable to more complicated compounds because the reaction conditions of CM are mild enough to tolerate a variety of functional groups. Furthermore, the method is operationally simple and applicable at a submilligram scale. Thus, we believe that our CM-based approach would be practically useful, especially for nonspecialized laboratories. The presented method is applicable to pure olefinic compounds, but there is some limitation on its use in olefin mixtures, such as biological extracts. Further studies and improvements to the method are needed for applications in analyzing a complex mixture of unsaturated hydrocarbons.

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[14] Other low-molecular-weight olefins (such as ethylene, isobutylene, 2,3-dimethyl-2-butene, acrylonitrile, and methacrylonitrile) would not meet the criteria, especially in terms of their boiling points and/or poor reaction efficacy.

[15] The reaction scale could be increased or reduced by a factor of up to 5 with no adverse affects.

[16] When catalyst **2** in PA was replaced by catalyst **4**, we observed an additional major peak that could be assigned to the product resulting from the CM reaction on the second double bond.

[17] For the removal of ruthenium byproducts by activated carbon, see: J. H. Cho, B. M. Kim, *Org. Lett.* **2003**, *5*, 531 – 533.

[18] The peak corresponding to the homodimerization product was detected, but it was negligible.

[19] The mass value of the homodimer also provides useful information regarding the position of the double bond. The mass difference between the homodimer and two molecules of olvanil is 252 Daltons. This value indicates that $C_{18}H_{36}$ was completely removed during the homodimerization. It also indicates that nine carbon atoms were abstracted from olvanil. Thus, the double-bond position of olvanil is ninth from the terminal methyl end.

