Identification and Analysis of a Gene from *Calendula officinalis* Encoding a Fatty Acid Conjugase

Xiao Qiu¹*, Darwin W. Reed, Haiping Hong¹, Samuel L. MacKenzie, and Patrick S. Covello

Research and Development, Bioriginal Food and Science Corporation, 102 Melville Street, Saskatoon, Saskatchewan, Canada S7J 0R1 (X.Q., H.H.); and National Research Council of Canada, Plant Biotechnology Institute, 110 Gymnasium Place, Saskatoon, Saskatchewan, Canada S7N 0W9 (D.W.R., S.L.M., P.S.C.)

Two homologous cDNAs, CoFad2 and CoFac2, were isolated from a *Calendula officinalis* developing seed by a polymerase chain reaction-based cloning strategy. Both sequences share similarity to FAD2 desaturases and FAD2-related enzymes. In *C. officinalis* plants CoFad2 was expressed in all tissues tested, whereas CoFac2 expression was specific to developing seeds. Expression of CoFad2 cDNA in yeast (*Saccharomyces cerevisiae*) indicated it encodes a Δ12 desaturase that introduces a double bond at the 12 position of 16:1(Z) and 18:1(Z) to calendic acid 18:3(8E, 10E, 12Z). The enzyme also has weak activity on the mono-unsaturates 16:1(Z) and 18:1(Z) producing compounds with the properties of 8,10 conjugated dienes.

Hundreds of different fatty acids, many of which have potential for industrial and pharmaceutical use, have been identified in nature (Smith, 1970). However, these fatty acids are mostly produced in the wild plant species or microorganisms that are not readily cultivated and cultured commercially. Conventionally oilseed crops have high yields and oil contents, but they produce a limited set of fatty acids, which usually contain less than three double bonds in their acyl chains.

Generally speaking, in polyunsaturated fatty acids (PUFA), double bonds tend to be methylene-interrupted and in the cis configuration. However, fatty acids containing conjugated double bonds with various stereochemical configurations do occur in bacteria, algae, and plants. In the marine algae *Bosisia orbigniana* and *Ptilota filicina*, a substantial proportion of the PUFA contain conjugated double bonds (Burgess et al., 1991; Wise et al., 1994). In plants, various conjugated linolenic acid isomers accumulate in seeds. Examples include α-eleostearic acid [18:3(9Z, 11E, 13E)] in *Momordica charantia* (Liu et al., 1997), punicic acid [18:3(9Z, 11E, 13Z)] in *Punica granatum* and *Cayaponia africana*, and jarcacic acid [18:3(8Z, 10E, 12Z)] in *Jacaranda mimosifolia* (Chisholm and Hopkins, 1967b; Hopkins and Chisholm, 1968). *Calendula officinalis* is an annual flowering plant that can accumulate more than 40% of calendic acid [18:3(8E, 10E, 12Z)] of the seed lipid fatty acids (Chisholm and Hopkins, 1967a). Although oils containing conjugated linolenic acids have potential value as drying oils, only α-eleostearic acid-containing oil from tung (*Aleurites fordii*) seeds is currently of commercial significance.

As compared with conjugated polyunsaturated acids, conjugated linoleic acids (CLAs) appear less commonly in nature. A few reports have documented the occurrence of this fatty acid in the foods derived from ruminant animals (Fritsche and Fritsche, 1998) and a number of anaerobic bacteria such as rumen bacterium *Butyryrivibrio fibrisolvens* (Kepler et al., 1966; Kepler and Tove, 1967). It is believed that CLAs are originally generated by rumen bacteria and then absorbed by the animal host (Pariza, 1997).

The diversity of fatty acids in nature is largely due to various combinations of the numbers and locations of double and triple bonds and other functional groups (hydroxyl and epoxy). These are produced by a family of structurally related enzymes (with three conservative His-rich motifs), including desaturases and their diverged forms such as hydroxylases, epoxygenases, acetylenases, and the so-called fatty acid conjugases (Lee et al., 1998; Shanklin and Cahoon, 1998; Cahoon et al., 1999). For microsomal enzymes in this category it is believed that they use fatty acids esterified to complex lipid as the substrate and accept electrons from an electron transport chain consisting of NAD(P)H, cytochrome b₅ reductase, and cytochrome b₅.

Based on the information that microsomal desaturases and related enzymes have similar primary structure, we undertook a PCR approach to clone genes that are involved in the biosynthesis of conjugated fatty acids in *C. officinalis*. Two unique cDNAs (CoFad2 and CoFac2) were identified. Expression of the two cDNAs in yeast (*Saccharomyces cerevisiae*) resulted in enzymatic accumulation of the conjugated fatty acids.
vealed that CoFAD2 is a Δ12 desaturase and CoFAC2 is a fatty acid conjugase that could convert the Δ9 double bond of linoleic acid and, to a lesser extent, of palmitoleic and oleic acids, into two conjugated double bonds at Δ8 and Δ10 position. To our knowledge this is the first example of identification of an enzyme that can produce CLAs.

RESULTS

*C. officinalis* is an annual flowering plant that has recently drawn scientific attention due to health claims of the essential oil in the flowers and the industrial potential of calendic acid in the seed oil. Calendic is the major fatty acid in the seeds, accounting for more than 40% of the total fatty acids. We are interested in the molecular basis for the biosynthesis of this special fatty acid.

Identification of a cDNA Coding for a Putative Fatty Acid Conjugase

To identify genes encoding conjugated double bond-forming enzymes in *C. officinalis*, a PCR-based cloning strategy was adopted. Sequencing of PCR products revealed three types of inserts related to desaturases. One had high sequence similarity to ω-3 desaturases (FAD3). The other two shared amino acid sequence similarity to various Δ12 desaturases (FAD2) and related enzymes, such as an acetylenase from *Crepis alpina* (Lee et al., 1998).

To isolate full-length cDNA clones the two types of Fad2-like inserts were used as probes to screen a cDNA library from developing seeds, which resulted in identification of several cDNA clones in each group. Sequencing identified two unique full-length cDNAs, *CoFad2* and *CoFac2*. *CoFad2* is 1,411 bp and codes for 383 amino acids with an M_r of 44,000. *CoFac2* is 1,310 bp in length and codes for 374 amino acids with a molecular mass of 43.6 kD. Sequence comparison revealed 46% amino acid identity between the two deduced proteins. The identity occurs all along the polypeptides with the highest among three conservative His-rich areas (Fig. 1).

Sequence comparisons indicate that CoFAD2 shares 73% to 89% amino acid identity with the Δ12 desaturases from various plants (Okuley et al., 1994; Lee et al., 1998; GenBank accession nos. AF188264 and AAC 31698). Whereas CoFAC2 shares approximately equal sequence identity (50%) both to FAD2 desaturases and to other FAD2-like fatty acid-modifying enzymes including FAD2 from *C. officinalis* (CoFAD2, this paper), Indian mustard (GenBank accession Q39287), and borage (GenBank accession no. AAC31698), the Δ12 acetylenase of *C. alpina* (Lee et al., 1998), the bifunctional enzyme (oleate 12-hydroxylase:12-desaturase) of *Lesquerella fendleri* (Broun et al., 1998), the 12,13-epoxygenase of *Crepis palaestina* (Lee et al., 1998), and fatty acid conjugases from *C. officinalis* (Fritsche et al., 1999), *Impatiens balsamina*, and *Momordica charantia* (Cahoon et al., 1999).

Phylogenetic analysis indicates that CoFAC2 distinguishes itself as one of the most deeply branching within the plant FAD2-like sequences (Fig. 2). Bootstrap analysis does indicate that this branching pattern is not particularly reliable and it is possible that CoFAC2 could cluster with other fatty acid conjugases, an epoxygenase, and an acetylenase. On the other hand, CoFAD2 is clearly grouped within a main branch of FAD2-like enzymes, which includes the FAD2s per se, as well as the *L. fendleri* bifunctional enzyme (Broun et al., 1998) and *Ricinus communis* hydroxylase (van de Loo et al., 1995). These results suggest the possible functions of CoFAD2 and CoFac2 as those of the extraplastidial Δ12 fatty acid desaturase and a fatty acid modifier likely to be involved in calendic acid biosynthesis, respectively.

Northern-Blot Analysis of CoFac2 and CoFad2

Northern-blot analysis indicated that the *CoFac2* was exclusively expressed in the developing seeds of *C. officinalis* (Fig. 3). It was not expressed in vegetative tissues such as leaves and in reproductive tissues such as flower buds. In contrast, *CoFad2* was expressed in all tissues tested such as leaves, flower buds, and developing seeds, but preferentially in flower buds and developing seeds. Expression patterns of the two genes were consistent with the pattern of calendic acid accumulation, which occurs only in seeds. In *C. officinalis* calendic acid accumulated only in seeds, whereas linoleic acid, the product of the Δ12 desaturase (CoFAD2), was present in all
three tissues examined, but the flower buds and developing seeds contain a higher amount of this fatty acid.

**Expression of CoFac2 and CoFad2 in Yeast**

To investigate the function of CoFac2 the full-length cDNA was expressed in the yeast strain AMY-2α in which the stearoyl-coenzyme A desaturase gene, ole1, is disrupted. The strain is unable to grow in minimal media without supplementation of mono-unsaturated fatty acids and allows for experimental control of the fatty composition of the yeast. In our experiments the strain was grown in minimal medium supplemented with 17:1(10Z), a non-substrate of CoFAC2, which enabled us to study the substrate specificity of the enzyme toward various substrates, especially mono-unsaturates. A number of possible substrates including 16:0, 16:1(9Z), 17:1(10Z), 18:0, 18:1(9Z), 18:1(9E), 18:1(11Z), 18:1(11E), 18:1(12Z), 18:1(15Z), 18:2(9Z, 12Z), 18:3(9Z, 12Z, 15Z), 20:0, 20:2(11Z, 14Z), and 22:1(13Z) were tested. As indicated in Figures 4 and 5 and Table I, only 18:2(9Z, 12Z) and, to a lesser extent, 16:1(9Z) and 18:1(9Z) were converted to conjugated fatty acids by the enzyme. For cultures supplemented separately with the three substrates, when gas chromatograms of fatty acid methyl esters (FAMEs) derived from strains expressing CoFac2 were compared with those for vector controls, extra peaks were detected as shown in Figure 4. These peaks were selectively ablated when a Diels-Alder reaction with 4-methyl-1,2,4-triazoline-3,5-dione (MTAD) was performed prior to gas chromatography (GC) analysis (data not shown). The sets of m/z peaks indicated in Figure 5 are highly diagnostic for the original double bond positions of the conjugated fatty acid analyte. Mass spectral (MS) analysis of the MTAD derivatives indicates that the products of 16:1, 18:1, and 18:2 conversion are 16:2(8, 10) and 18:2(8, 10) (Fig. 5) and 18:3(8, 10, 12) (data not shown). Assignment of the product of 18:1(9) conversion is also supported by the agreement of its GC peak retention
As for the function of CoFad2, the expression of it in the wild-type yeast strain INVSc2 indicated that the encoded enzyme is a $\Delta_{12}$ desaturase that introduces a double bond at position 12 of 1:1(9Z) and 18:1(9Z) (data not shown).

**DISCUSSION**

In this report we described the identification and characterization of two homologous cDNAs, CoFac2 and CoFad2, from *C. officinalis*. Both products have sequence similarity to the FAD2 desaturases and related enzymes from plants. CoFAD2 has higher amino acid identity to the FAD2 desaturases (approximately 80%), whereas CoFAC2 has approximately equal sequence identity (approximately 50%) to both FAD2 desaturases and FAD2-related enzymes, including the $\Delta_{12}$ acetylenase of *C. alpina* (Lee et al., 1998), a bifunctional enzyme (oleate 12-hydroxylation: 12-desaturase) of *L. fendleri* (Broun et al., 1998), an epoxygenases from *C. palaeastina* (Lee et al., 1998), fatty acid conjugases from *C. officinalis* (Fritsche et al., 1999), *I. balsamina*, and *M. charantia* (Cahoon et al., 1999). Expression of CoFad2 cDNA in yeast indicated it encodes a $\Delta_{12}$ desaturase, whereas expression of CoFac2 in yeast revealed that the encoded enzyme produced conjugated linoleic and linolenic acids from 18:1(9Z) and 18:2(9Z, 12Z) substrates, respectively.

The name “conjugase” was previously coined to refer to enzymes that are responsible for introducing conjugated double bonds into acyl chains. Two conjugases from *I. balsamina* and *M. charantia* were found to be able to convert the $\Delta_{12}$ double bond of linoleic acid into two conjugated double bonds at the 11 and 13 positions, resulting in the production of conjugated linolenic acid [18:3(9Z, 11E, 13E)]. Expression of CoFac2 in yeast showed that this “conjugase” could convert $\Delta^9$ double bonds of 16:1(9Z), 18:1(9Z), and 18:2(9Z, 12Z) into two conjugated double bonds at the 8 and 10 positions to produce their corresponding conjugated fatty acids.

Two major routes to the biosynthesis of conjugated fatty acids have been elucidated. Isomerization of a common fatty acid into its conjugated counterpart without the introduction of additional double bonds was first described during biohydrogenation of linoleic acid by anaerobic rumen bacteria (Polan et al., 1964). In marine algae a recently identified enzyme can isomerize several PUFA into their corresponding conjugated polyenoic acids (Wise et al., 1994, 1997). The process is strictly an isomerization; there is no oxidized intermediate or net desaturation involved.

In plants the mechanism underlying biosynthesis of conjugated linolenic acids was studied by radiolabeling (Crombie and Holloway, 1985; Liu et al., 1997). Kinetics of the time course of metabolism of the radiolabeled precursors indicated linoleic acid esterified to phosphatidylcholine is an intermediate precursor of conjugated linolenic acid, implying that...
there is desaturation involved. Substrate specificity studies of CoFAD2, along with that of conjugases from *I. balsamina* and *M. charantia* (Cahoon et al., 1999), favor the hypothesis that conjugated fatty acids in plants are produced by a process similar to desaturation, which can result in introduction of one additional double bond in the existing fatty acid substrate. Crombie and Holloway (1985) previously observed that during conversion of linoleic acid to calendic acid in *C. officinalis* developing seeds, there is no loss of labeled hydrogens at C-9, C-10, C-12, and C-13, but there is a loss of a hydrogen from C-8 and C-11. Thus, Fritsche et al. (1999) speculated that the *C. officinalis* fatty acid conjugase could abstract hydrogens at carbon 8 and 11 positions, resulting in two conjugated double bonds in the 8 and 10. Two genes have now been cloned from *C. officinalis* whose products appear to catalyze the production of calendic acid. However, it is still not clear whether both cloned enzymes actually act via an “8,11 desaturation” mechanism.

It was unexpected that 18:1 (9) acts as a substrate, albeit a weak one, for CoFAC2 giving rise to conjugated linoleic acid [18:2(8, 10)] in yeast. CLA is a newly recognized nutraceutical compound that has recently drawn the attention of the pharmaceutical and nutraceutical industries because of its various physiological effects in animals and humans (Hau mann, 1996; Ip, 1997; Pariza, 1997). Dietary CLA (two major isomers: 9Z, 11E and 10E, 12Z) was shown to reduce the development of atherosclerosis in rabbits (Lee et al., 1994) and to inhibit development of various cancers in model animals (Pariza et al., 1999). Feeding CLAs at low concentration (0.5% of diet) to rodents can enhance immune function (Miller et al., 1994). In addition, CLAs were recently found to decrease fat composition and increase lean body masses and to improve feed efficiency in chickens and pigs (Park et al., 1997). With the realization of the benefits of CLAs, market demand for the product is growing. There is, unfortunately, no rich natural source for CLAs. Although some animal foods such as dairy products and meat derived from ruminants contain CLAs, the proportion is low. Linoleic acid can be converted to CLA by chemical methods (Berdeaux et al., 1998; Chen et al., 1999). However, CLA derived from the chemical process is a mixture of several isomers. The two major isomers (9Z, 11E and 10E, 12Z) in about equal proportions account for about 80% of the product. The rest are other CLA isomers.

CLA produced by CoFAC2 in yeast is an unusual isomer with two conjugated double bonds at the 8 and 10 positions. The stereochemistry of the product remains to be determined. It is likely that it is 8E and 10E, since calendic acid [18:3(8E, 10E, 12Z)] is also a product of the enzyme in yeast.

The finding that CoFAC2 can use oleic substrate to synthesize the CLA has opened up a question regarding the potential uses: does this CLA isomer have any physiological effects on human and animal as common CLA does? To answer the question, preparation of large amounts of the isomer is the first essential step since feeding experiments and clinical trials would consume a large amount of the fatty acid. If the efficiency of conversion of 18:1 to CLA could be improved, it may be possible to produce the 8,10 isomer commercially in genetically modified organisms.

### MATERIALS AND METHODS

#### Plant Materials

*Calendula officinalis* was grown in a growth chamber at 22°C with a 16-h photoperiod at a photon flux density of 150 to 200 µE m⁻² s⁻¹. The developing seeds at 15 to 30 d after flowering were collected. The embryos were dissected from seeds and used for RNA isolation.

#### Construction and Screening of cDNA Library

The total RNA was isolated from developing embryos according to Qiu and Erickson (1994). The cDNA library was constructed from the total RNA. The first strand cDNA was synthesized by superscript II reverse transcriptase from Gibco-BRL (Gaithersburg, MD). The second strand cDNA was synthesized by DNA polymerase I from Stratagene (La Jolla, CA). After size fractionation, cDNA inserts larger than 1 kb were ligated into λ Uni-Zap XR vector (Stratagene). The recombinant λ DNAs were then packaged with Gigapack III Gold packaging extract (Stratagene) and plated on NZ amine-yeast extract plates. The resulting library represented more than 8 x 10⁶ independent clones. Screening of the cDNA library was performed according to standard methods (Sambrook et al., 1989).

### Table I. Conversion of exogenous fatty acids by the yeast strain AMY-2α/pCoFac2

See “Materials and Methods” for culture conditions. Values are the means and SDs (in parentheses) of three experiments. For control experiments using the AMY-2α/pYES2 strain, no significant peaks were detected at the retention time of the desaturation product.

<table>
<thead>
<tr>
<th>Substrate Supplied</th>
<th>Substrate Accumulation</th>
<th>Product</th>
<th>Product Accumulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:1(9)</td>
<td>45 (12)</td>
<td>16:2 (8,10)</td>
<td>0.29 (0.04)</td>
</tr>
<tr>
<td>18:1(9)</td>
<td>42 (10)</td>
<td>18:2 (8,10)</td>
<td>0.09 (0.02)</td>
</tr>
<tr>
<td>18:2(9, 12)</td>
<td>18 (3)</td>
<td>18:3 (8E, 10E, 12Z)</td>
<td>0.56 (0.3)</td>
</tr>
</tbody>
</table>

Copyright © 2001 American Society of Plant Biologists. All rights reserved.
Reverse Transcriptase-PCR

For reverse-transcriptase experiments the single strand cDNA was synthesized by superscript II reverse transcriptase (Gibco-BRL) from total RNA and was then used as the template for PCR reaction. Two degenerate primers (the forward primer: GCXAC/TGAC/A/GTGC/TGGXAC/TC/GA and the reverse primer: CATXGTXG/CA/TG/AAAXAG/AG/ATGG/ATG) were designed to target the conserved His-rich domains of desaturases. The PCR amplification consisted of 35 cycles with 1 min at 94°C, 1.5 min at 55°C, and 2 min at 72°C followed by an extension step at 72°C for 10 min. The amplified products from 400 to 600 bp

Figure 5. GCMS EI spectra of the MTAD derivatives of novel fatty acids in AMY-2α/pCoFac2 cultures supplemented with 16:1(9Z), (A) and 18:1(9Z), (B). The structures assigned to the derivatives are shown with asterisks indicating the original position of the double bonds in the fatty acid. The pairs of peaks with m/z values 236 and 308 in A and 264 and 308 in B are diagnostic for the loss of R₁ and R₂ fragments, respectively, for 16:2(8, 10) and 18:2(8, 10) derivatives.
were isolated from agarose gel and purified by a kit (Qiaex II gel purification, Qiagen, Valencia, CA), and subsequently cloned into the TA cloning vector pCR 2.1 (Invitrogen, Carlsbad, CA). The cloned inserts were then sequenced by PRISM DyeDeoxy Terminator Cycle Sequencing System (Perkin Elmer/Applied Biosystems, Foster City, CA).

Phylogenetic Analysis

For phylogenetic analysis, predicted amino acid sequences were aligned using CLUSTALW (version 1.60; Thompson et al., 1998) with the default parameters, including gap open and extension penalties of 10 and 0.05, respectively, for pairwise and multiple alignments. The BLOSUM 30 protein weight matrix was used for pairwise alignments and the BLOSUM series for multiple alignments. CLUSTALW was used to determine dendrograms representing a neighbor-joining analysis of sequence distances. Bootstrap analysis was performed with 1,000 iterations and visualized with the TreeView program (Page, 1996).

Northern-Blot Analysis

For northern-blot analysis, 7 μg of total RNAs isolated from flower buds, leaves, and developing seeds of C. officinalis as described above were fractionated in a formaldehyde-agarose gel. After electrophoresis RNAs were transferred to Hybond membrane (Amersham Pharmacia, Uppsala) using 10× SSC transferring solution and were then fixed to the membrane by UV crosslinking. Filter-bound RNAs were then hybridized with the radiolabeled cDNA probes at 68°C for 1 h in Quickhyb (Stratagene). After hybridization the blots were washed once at 2× SSC and 1% (w/v) SDS, and once at 65°C for one-half an hour with a solution of 0.1× SSC and 0.1% (w/v) SDS.

Expression of CoFad2 and CoFac2 in Yeast (Saccharomyces cerevisiae)

The open reading frames of CoFad2 and CoFac2 were amplified by PCR using the Precision Plus enzyme (Stratagene) and cloned into a TA cloning vector (pCR 2.1, Invitrogen). Having confirmed that the PCR products were identical to the original cDNAs by sequencing, the fragments were then released by a BamHI- EcoRI double digestion and inserted into the yeast expression vector pYES2 (Invitrogen) under the control of the inducible promoter GAL1.

Yeast strains InvSc2 (Invitrogen) and AMY-2α [the genotype: MATα, CYTb5, ole1(ΔAslEII):LEU2, trp1-1, can1-100, ura3-1, ade2-1, HIS3; Mitchell and Martin, 1995] were transformed with the expression constructs using the lithium acetate method and transformants were selected on minimal medium plates lacking uracil (Gietz et al., 1992; Covello and Reed, 1996).

Transformants were first grown in minimal medium lacking uracil and containing Glc (CM-ura, Ausubel et al., 1995) at 28°C. After overnight culture the cells were spun down, washed, and resuspended in distilled water. Minimal medium with 2% (w/v) Gal replacing Glc, and with or without 0.3 mM substrate fatty acids in the presence of 0.1% (w/v) Tergitol was inoculated with the yeast transformant cell suspension and incubated at 20°C for 3 d followed by 15°C for 3 d. For the AMY2α strain media were supplemented with 0.3 mM 17:1(10Z) and 0.1% (w/v) Tergitol.

Fatty Acid Analysis

Yeast cultures were pelleted by centrifugation (4,000g, 10 min.) and pellets were washed with 10 mL of 1% (w/v) Tergitol solution and 2× 10 mL of water. The yeast pellet was dried under vacuum at ambient temperature. To the dried pellet in a glass culture tube was added 1 mL of methanol and the pellet was dispersed using a high speed homogenizer. To this mixture was added 2 mL of 0.5 M sodium methoxide in methanol. The tube was flushed with nitrogen, sealed, and heated to 50°C for 1 h. The cooled mixture was extracted with 2× 2 mL of hexane. The pooled hexane was washed with 2 mL of water and was concentrated under N2 for GC or GC/MS analysis.

FAME analysis was carried out using a gas chromatograph (6890, Hewlett-Packard, Palo Alto, CA) equipped with a DB-23 fused silica column (30 m×0.25 mm i.d., 0.25-μm film thickness; J&W Scientific, Fulsom, CA) with a temperature program of 180°C for 1 min, 4°C/min to 240°C, hold for 15 min.

For conjugated polyene analysis, FAME were derivatized with MTAD (Dobson, 1998). One hundred microliters of a dilute solution of MTAD (~1 mg/mL, slight pink color) in CHCl3 at 0°C was added to dry FAME from yeast cells with agitation for 5 to 10 s. A dilute solution of 1,3-hexadiene (excess) was then added to neutralize reactants (removal of color). The tube was dried under nitrogen and the residue was re-dissolved in CHCl3.

GC/MS analysis was performed in standard EI mode using a Fisons VG TRIO 2000 mass spectrometer (VG Analytical, Manchester, UK) controlled by Masslynx version 2.0 software, coupled to a GC 8000 Series gas chromatograph. For FAME analysis, a DB-23 column was used with the temperature program described above. For MTAD derivative analysis, a DB-5 column (60 m×0.32 mm i.d., 0.25-μm film thickness, J&W Scientific) that was temperature-programmed at 50°C for 1 min, increased at 20°C/min to 160°C, then 5°C/min to 350°C and held for 15 min.

In some experiments, C. officinalis oil extracted from seeds or a mixture of CLAs (Sigma, St. Louis) was used as the standard.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Ron Wilen for providing C. officinalis seeds, Dr. Charles Martin for providing yeast AMY-2α mutant strain, and Stephen Ambrose for GC/MS analysis.

Received June 6, 2000; returned for revision July 20, 2000; accepted October 12, 2000.
LITERATURE CITED


Dobson G (1998) Identification of conjugated fatty acid bodies by gas chromatography-mass spectrometry of 4-methyl-1,2,4-triazoline-3,5-dione adducts. JAOCs 75(2): 137–142


Copyright © 2001 American Society of Plant Biologists. All rights reserved.

Downloaded from www.plantphysiol.org on July 16, 2017.

