One-Step Methodology for the Synthesis of FA Picolinyl Esters from Intact Lipids

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ABSTRACT: Picolinyl derivatives are used for structural determination of FA by GC–MS. Although they provide reliable diagnostic fragments, the usual multistep methodologies applied for their preparation require TAG hydrolysis or acid chloride formation prior to picolinyl synthesis. These reaction conditions may result in the presence of artifact molecules in the samples and thus compromise analytical quality and accuracy. To address these problems, a rapid, simple and quantitative methodology for the synthesis of FA picolinyl esters from intact lipids was developed. It involves their transesterification under base-catalyzed conditions using 3-potassiooxamethylpyridine in methylene chloride. The catalyst was prepared by proton exchange between potassium *tert*-butoxide and anhydrous 3-hydroxymethylpyridine. Mild reaction conditions allowed complete derivatization of TAG and phospholipids in 2 min at room temperature, and of FAME in 15 min at 45°C. The proposed procedure, which can be used on a routine basis, was applied to *Ipomoae imperialis* seed lipids and used to confirm occurrence of γ-linoleic acid at a level of 0.9%.

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KEY WORDS: Alkoxide, fatty acid picolinyl ester, gas–liquid chromatography, *Ipomoae* seed oil, γ-linolenic acid, mass spectrometry, mass spectroscopy, potassium *tert*-butoxide, transesterification.

Derivatization is a necessary step prior to structural determination of FA by GC–MS. However, mass spectra of FAME, the usual derivatives for GLC–FID analysis, are devoid of sufficient information for identification of structural isomers. This is mainly due to the high sensitivity of the carboxyl group to fragmentation and to double bond migration (1,2). However, stabilization of the carboxyl group by the formation of a derivative containing a nitrogen atom results in mass spectra that allow structural determination for most FA (1). A first such derivative used in GC–MS analysis was pyrrolidide (3), but structural determination of FA by this technique is now usually performed using picolinyl and 4,4-dimethyloxazoline derivatives. Indeed, these FA derivatives provide diagnostic fragments that allow accurate structure determination (1). However, the usual multistep methodologies for the synthesis of picolinyl esters require hydrolysis of the lipid sample prior to final derivatization, either by condensation of the formed acid chloride with 3-hydroxymethylpyridine (1) or through an imidazole intermediate (4). The latter methodology was developed for the analysis of sensitive FA containing polyethylenic bond systems or an epoxy group. Both procedures give accurate qualitative and quantitative results but require long reaction times, which do not always lead to complete derivatization, and moderate to high temperatures that may result in artifacts.

The aim of this study was to develop a short, simple, and quantitative procedure for the synthesis of FA picolinyl esters in mild conditions and from O-acetylated acids. The proposed methodology was applied to confirm occurrence of γ-linoleic acid in *Ipomoae imperialis* seed oil.

MATERIALS AND METHODS

Standards and reagents. Potassium *tert*-butoxide (1.0 M in THF) and 3-hydroxymethylpyridine were purchased from Aldrich Chemicals (Milwaukee, WI). Monopalmitin, dipalmitin, tripalmitin, and FAME standards (#GLC-60, C_4 to C_{20} acids) were obtained from Nu-Chek-Prep (Elysian, MN). Phosphatidylcholine standard from egg yolk was purchased from Sigma Chemical (St. Louis, MO).

Synthesis of picolinyl esters and optimization of reaction conditions. Transesterification of tripalmitin, FAME, and phosphatidylcholine into picolinyl derivatives was performed as follows: A solution of potassium *tert*-butoxide in THF (100 μL, 1.0 M) was added to 3-hydroxymethylpyridine (200 μL). After homogenization, the lipid sample (10 mg) in dry dichloromethane (1 mL) was added to the reagent, and the mixture was held at 22 or 45°C for 2 to 30 min in a closed vial. After cooling to room temperature, as needed, a dilute (2.5%) aqueous solution of sodium bicarbonate (1 mL) was added, and the organic phase was collected, dried over anhydrous sodium sulfate, filtered, and analyzed. Progression of the reaction was monitored by GLC on a Hewlett-Packard gas chromatograph (model 6890 Series II; Palo Alto, CA) equipped with an FID and connected to a computer with a ChemStation, using a 65% phenyl-methylpolysiloxane RTX-65 TG (Restek, Bellefonte, PA; 30 × 0.25 mm i.d., 0.10 μm film thickness) capillary column. The injection (split mode)

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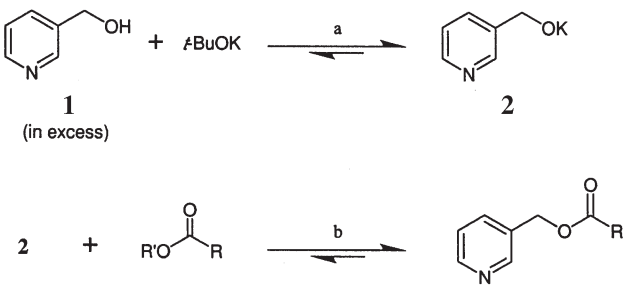
and detection (flame-ionization) were performed at 400°C, with an oven temperature of 190°C isothermal for 8 min, increased to 360°C at 10°C min^{-1}, and isothermal for 10 min at this temperature. The inlet pressure of the carrier gas (H_2) was 100 kPa. Under these conditions, picolinyl palmitate and mono-, di-, and tripalmitin are fully resolved. Glycerides were identified using standards.

Oil extraction. Mature dry seeds (2 g) of morning glory (*Ipomoae imperialis*), purchased from a local seed retailer, were finely ground in a mortar, and the oil was extracted with hexane using a Soxhlet apparatus. The extract was filtered and dried over anhydrous sodium sulfate, and the oil was recovered by evaporation of the solvent under vacuum at 30°C in a rotary evaporator (23% yield). The oil was stored under N_2 at –35°C until further use.

GC of FAME. Methylation of FA in the oil sample was carried out in a sealed tube with 0.4 N sodium methoxide in methanol. Analysis of FAME was performed with the same apparatus as above. Sample volumes (1.0 μL) in hexane were injected on a BPX-70 (equivalent to 70% cyanopropyl; SGE, Melbourne, Australia; 25 m × 0.22 mm i.d., 0.25 μm film thickness) capillary column. The injector (split ratio 25:1) and detector temperatures were maintained at 250°C, while the oven temperature was 210°C isothermal for 30 min. Hydrogen was used as carrier gas under a constant head pressure of 100 kPa.

GC–MS analysis of picolinyl esters. FA picolinyl esters were synthesized according to the above procedure at 22°C, for 2 min. They were analyzed by GC–FID under conditions similar to those above, and by GC–MS (Hewlett-Packard model 6890 Series II gas chromatograph attached to an Agilent model 5973N selective quadrupole mass detector; Palo Alto, CA) under an ionization voltage of 70 eV at 250°C, and connected to a computer with a Hewlett-Packard ChemStation. The injector, in split mode (25:1), and the interface temperatures were maintained at 250°C, and He was used as carrier gas under constant flow (1 mL min^{-1}). GLC separation was performed on a BPX-70 capillary column (SGE; 60 m × 0.25 mm i.d., 0.25 μm film thickness) with an oven temperature of 200°C isothermal for 10 min, increased to 240°C at 5°C min^{-1} for 20 min and then increased to 260°C at 5°C min^{-1}.



(a) THF, 22°C, 2 min; (b) THF-CH_2Cl_2, **1**, 22–40°C, 2–15 min, >99%

SCHEME 1

RESULTS AND DISCUSSION

Synthesis of picolinyl esters. The proposed new methodology for the synthesis of FA picolinyl ester derivatives for GC–MS analysis consists of base-catalyzed transesterification of intact lipids using 3-potassiooxamethylpyridine **2** as catalyst (Scheme 1). Mild reaction conditions (22–45°C), short reaction times (2–15 min), and direct transesterification are the principal advantages of this methodology compared to the usual procedures. The first step consists of the preparation of the alkoxide catalyst by proton exchange with a stronger base, potassium *tert*-butoxide, the conjugated base of *tert*-butanol, a tertiary alcohol, in THF and 3-hydroxypyridine **1**, a primary alcohol. It

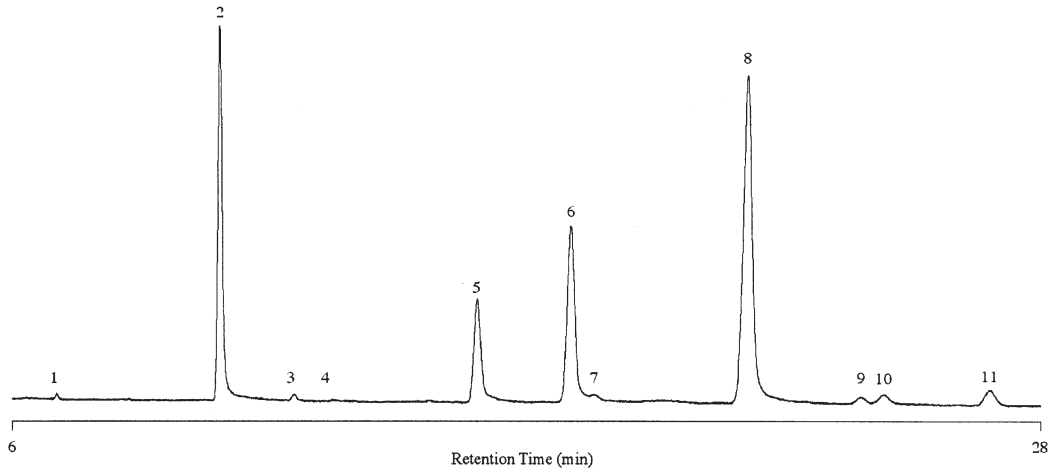


FIG. 1. GLC of FA picolinyl esters prepared from *Ipomoae imperialis* seed oil and analyzed on a 25-m cyanoalkylpolysiloxane capillary column (see Materials and Methods for detailed conditions). Peak identification: (1) 14:0; (2) 16:0; (3) *cis*-9 16:1; (4) 17:0; (5) 18:0; (6) *cis*-9 18:1; (7) *cis*-11 18:1; (8) *cis*-9,*cis*-12 18:2; (9) *cis*-6,*cis*-9,*cis*-12 18:3; (10) 20:0; (11) *cis*-9,*cis*-12,*cis*-15 18:3.

TABLE 1
FA Composition (wt%) of Oil from Seeds of *Ipomoae imperialis* from Methyl and Picolinyl Ester Derivatives[a]

FA	Derivative	
	Methyl[b]	Picolinyl[c]
14:0	0.2	0.2
16:0	21.1	22.1
cis-9 16:1	0.4	0.5
17:0	0.1	0.1
18:0	10.7	10.6
cis-9 18:1	19.5	19.2
cis-11 18:1	0.4	0.4
cis-9,*cis*-12 18:2	42.3	41.8
cis-6,*cis*-9,*cis*-12 18:3	0.9	0.9
cis-9,*cis*-12,*cis*-15-18:3	2.4	2.2
20:0	1.7	1.6
Others	0.3	0.4

[a]Values are means of triplicate analysis; wt(%) of FA.
[b]FAME.
[c]FA picolinyl ester.

is well known that pK_a values for tertiary alcohols are 2 to 3 units higher than those for primary alcohols. The lipid sample in methylene chloride (the use of hexane results in base precipitation) is added to the catalyst solution for derivatization. We found it more convenient to use a solution of potassium *tert*-butoxide for catalyst formation than metallic potassium or sodium, since solutions of potassium *tert*-butoxide are commercially available. This reagent is indeed sensitive to moisture; therefore, dry solvents and reagents should be used. For optimization of the derivatization parameters, solutions of tripalmitin, phosphatidylcholine, and FAME in methylene chloride were added to solutions of **2** in THF, and rates of transesterification were monitored at different temperatures by GLC. Transesterification of TAG and phospholipids can be efficiently performed at room temperature in less than 2 min, whereas transesterification of FAME is complete in 15 min at 40–50°C. While the proposed procedure will perform transesterification on O-acylated acids, it cannot be applied to FFA. In this case, the usual methods should be used (4,5).

Analysis of I. imperialis *seed oil.* The oil content in the seeds of *I. imperialis*, commonly known as morning glory, was 23%. A highly polar BPX-70 capillary column was used for FA separation for both methyl and picolinyl ester derivatives (Fig. 1). The comparative FA composition, based on methyl and picolinyl derivatives, presented in Table 1 confirms the quantitative values for picolinyl esters using the newly developed procedure. The major FA derived from the oil were linoleic, palmitic, oleic, and stearic acids, with FAME values of 42.3, 21.1, 19.5, and 10.7%, respectively. The oil also contained lower levels of two trienoic FA, α- and γ-linolenic acids. The mass spectrum of picolinyl γ-linolenate (Fig. 2) shows the typical ion fragmentation pattern of a monomethylene-interrupted double bond system (ion fragments at *m/z* = 298, 272, 258, 232, 218, and 192) and a molecular ion at *m/z* = 369, in agreement with literature data (6), thus confirming the occurrence of this FA.

In this paper, we have demonstrated that picolinyl ester derivatives of FA can easily be prepared using base catalysts, in a similar manner to methyl ester derivatization by sodium methoxide in methanol. The use of such an alkoxide containing a nitrogen atom, and prepared from potassium *tert*-butoxide, is not limited to the 3-hydroxymethylpyridine presented in this paper, and other FA derivatives synthesized for specific purposes can be prepared by following the same procedure. The methodology was applied to confirm the occurrence of low quantities of γ-linolenic acid in morning glory (*I. imperialis*) seed oil.

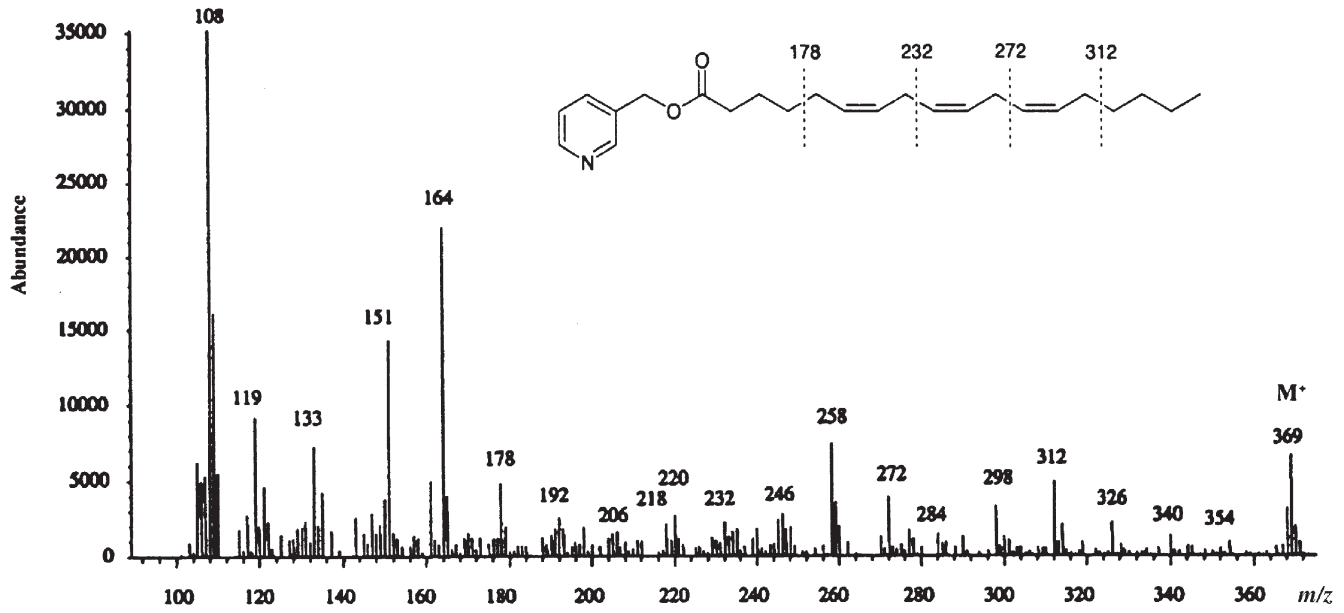


FIG. 2. Mass spectrum of picolinyl ester derivative of *cis*-6,*cis*-9,*cis*-12 18:3 FA from *Ipomoae imperialis* seed oil.

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REFERENCES

1. Christie, W.W., Gas Chromatography–Mass Spectrometry Methods for Structural Analysis of Fatty Acids, *Lipids 33*:343–353 (1998).
2. Spitzer, V., Structure Analysis of Fatty Acids by Gas Chromatography—Low Resolution Electron Impact Mass Spectrometry of Their 4,4-Dimethyloxazoline Derivatives—A Review. *Prog. Lipid Res. 35*:387–408 (1997).
3. Andersson, B.A., Mass Spectrometry of Fatty Acid Pyrrolidides, *Prog. Chem. Fats Other Lipids 16*:279–308 (1978).
4. Harvey, D.J., Picolinyl Esters as Derivatives for the Structural Determination of Long-Chain Branched and Unsaturated Fatty Acids, *Biomed. Mass Spectrom. 9*:33–38 (1982).
5. Balazy, M., and A.S. Nies, Characterization of Epoxides of Polyunsaturated Fatty Acids by Mass Spectrometry *via* 3-Pyridinylmethyl Esters, *Biomed. Environ. Mass Spectrom. 18*:328–336 (1989).
6. Wretsenjo, I., L. Svensson, L., and W.W. Christie, Gas Chromatographic–Mass Spectrometric Identification of Fatty Acids in Borage Oil Using the Picolinyl Ester Derivatives, *J. Chromatogr. 521*:89–97 (1990).

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