Configurational Purity of Lesquerolic Acid

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ABSTRACT: Conversion of the methyl ester of lesquerolic acid, (*R*)-14-hydroxy-*Z*-11-eicosenoic acid, to a carbamate with (*S*)-1-(1′-naphthyl)ethylisocyanate gave a single diastereomer. Comparisons of elution orders of the relevant diastereomers obtained from the racemic ester and from a sample of lesquerolic acid synthesized from ricinoleic acid, (*R*)-12-hydroxy-*Z*-9-octadecenoic acid, were consistent with the original assignment of (*R*)-configuration for the longer-chain acid. Although the configuration had not been in doubt, this work demonstrates the configurational purity of the lesquerolic acid.
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KEY WORDS: Configuration, ^{1}H NMR (of diastereomers), HPLC (of diastereomers), lesquerolic acid, ricinoleic acid.

We recently published a method for determining the configuration of hydroxy fatty acid (HFA) methyl esters in which the asymmetric center was a secondary alcohol (1). The method requires the formation of a carbamate with the available chiral derivatizing agent (*R*)- or (*S*)-1-(1′-naphthyl)ethylisocyanate. The diastereomers are generally separable by (achiral) silica gel high-performance liquid chromatography (HPLC), and the ^{1}H nuclear magnetic resonance (NMR) shifts of the carbomethoxy methyl groups were distinguishable in the cases examined. Assignments of configuration in that work were based on the configurations of ricinoleic and isoricinoleic acids, which had been made previously by asymmetric synthesis. These assignments were consistent with the known solution conformation preferences of aliphatic carbamate diastereomers that are responsible for the observed NMR shift differences.

Lesquerolic acid (20:1^{11}–OH^{14}) is a homologue of ricinoleic acid in which the homoallylic unit is two carbons further removed from the carboxyl group. The optical rotation for the two compounds are both (+)(2), and alkaline cleavage of both acids led to weakly levorotatory 2-octanol (3). In addition, the chemical synthesis of (+)-lesquerolic acid from (+)-ricinoleic acid was accomplished (4), which led to the assignment of the (*R*)-configuration to the longer-chain acid. We wished to augment the data by determining the configurational purity of a sample of the acid by using the method of diastereomer formation. Although the ricinoleic acid of castor oil was essentially configurationally pure, samples of isoricinoleic proved variable (5). Material from seeds of *Holarrhena antidysenterica*, for example, had the (*S*)-configuration (90:10), while that from seeds of morning glory was essentially 100% (*R*). Chromatographic and spectral methods for determining configurational purity are particularly helpful when rotations are low and/or the sample is not completely pure. Moreover, it seemed important to document configurationally pure HFAs; these have the potential for high value use as chiral synthons in organic synthesis.

MATERIALS AND METHODS

All reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI), and solvents were reagent-grade or better. Tetrahydrofuran (THF) was distilled from lithium aluminum hydride (LAH) prior to use, and hexamethylphosphoramide (HMPA) and dimethylformamide (DMF) were dried over 13Å molecular sieves. The sample of lesquerella oil employed for this work was extracted from the seeds of *Lesquerella fendleri* (family Cruciferae or, equivalently, Brassicaceae) and refined (6). Gas–liquid chromatography (GLC) was performed with a Supelcowax column (0.25 mm i.d. × 30 m) from Supelco (Bellefonte, PA) operated at 260°C in a Chrompack-Packard Model 438A chromatograph (Avondale, PA), operating with helium carrier gas at 18 cm s^{-1} and a 50:1 split ratio. Thin-layer chromatography (TLC) was performed on plates of Adsorbosil from Alltech Associates, Inc. (Deerfield, IL) with various ratios of ethyl acetate and hexane (EA–H). HPLC was performed with a Spectraphysics (Piscataway, NJ) SP8800 pump, SP8480XR scanning ultraviolet detector set at 280 nm, and an SP4290 integrating recorder with a Supelcosil LC-Si analytical column (4.6 mm × 25 cm) from Supelco and 95:5:1 (H/EA/THF) as solvent at a flow rate of 1.0 mL/min. Infrared (IR) data were recorded with a Perkin Elmer Model 1310 spectrophotometer (Norwalk, CT) from 0.5% solutions in CCl_4. Mass spectra were obtained with a Hewlett-Packard HP-5995 GC/mass spectrometer (MS) system (Sterling, VA) by using the direct probe. ^{1}H and ^{13}C NMR were obtained with a JEOL-JNM-GX 400 Fourier Transform spectrometer (Peabody, MA) with deuteriochloroform as the solvent and tetramethylsilane as internal standard. Only diagnostic signals are reported for ^{13}C NMR. Flash are chromatography was performed with 60Å 230–400 mesh silica gel (Aldrich Chemical Co.).

Preparation of racemic methyl lesquerolate. The methyl ester of natural lesquerolic acid (84.5% pure by GLC: 240°C, 13.7 min), 0.90 g, was dissolved in 25 mL ether and cooled

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in an ice bath. Jones reagent (7), 2 mL, was added dropwise until the dichromate color persisted. The organic phase was washed with 5% $NaHSO_3$, dried ($MgSO_4$), and concentrated. The oily ketoester was dissolved in 25 mL methanol and cooled in an ice bath. The mixture was treated with $NaBH_4$ (0.5 g) and then stirred for 2 h without external cooling. The reaction mixture was diluted with H_2O and extracted with ether. The ethereal layer was washed with H_2O, dried ($MgSO_4$), and concentrated. The material was identical by GLC to the original ester and was used directly.

Preparation of carbamates. The procedure followed was published earlier (1). The carbamates were well separated (Fig. 1): $k' = 3.74$ and 4.08; $\alpha = 1.09$. The ^1H NMR shifts for $-CO_2CH_3$ (Fig. 2) were $\delta = 3.263$ (HPLC #1) and 3.252 (HPLC #2).

Preparation of (R)-14-(t-butyldimethylsiloxy)-Z-9-octadecenoic acid, methyl ester, **1**. Methyl ricinoleate (10.0 g, 32.1 mmol) was combined with imidazole (5.44 g, 80.2 mmol) and *t*-butyldimethylsilyl chloride (5.8 g, 38.5 mmol) in 20 mL of dry DMF under nitrogen. The mixture was stirred at 35°C for 16 h and then was worked up in the usual fashion by extracting into hexane and washing with water. The crude product was passed through a 45-mm i.d. flash chromatography column (overloading) with 5% EA–H, and we collected 50-mL fractions. Fractions 2–6 were pure (TLC: 45% EA–H, $R_f = 0.65$) and were combined to give **1**; 11.36 g, 83.1%: IR 1735, 1035b cm^{-1}; ^1H NMR δ5.39 (2H, *m*, HC=CH), 3.65 (4H, OCH_3, OCH), 2.29 (2H, *bt*, $CH_2CO_2CH_3$), 2.17 and 2.00 (4H, *m's*, CH_2C=C), 1.61 (2H, *m*, $CH_2CH_2CO_2CH_3$), 1.29 (18.3H, CH_2s), 0.88 (12H, *t*Bu and RCH_3), 0.037 (6H, *s*, $SiCH_3$) ppm; ^{13}C NMR δ163.77 (C=O), 131.28 and 125.97 (C=C), 72.42 (R_2CHOSi), 51.38 (OCH_3), 26.07 (*t*Bu), -4.36 ($SiCH_3$) ppm.

Preparation of (R)-1-Bromo-14-(t-butyldimethylsiloxy)-Z-9-octadecene, **2**. The silylated ester (11.0 g, 25.8 mmol) was reduced by adding it as an ether solution (10 mL) dropwise to a stirred suspension of LAH (1.0 g) in ether (30 mL) with cooling in an ice bath. The mixture was stirred without external cooling for 1 h and then carefully worked up by further addition of wet ether and 1.25N NaOH with ice-cooling. The mixture was suction-filtered, and the ethereal layer was washed with water, dried ($MgSO_4$), and concentrated to give the crude product (TLC 30% EA–H, $R_f = 0.48$): IR 3630, 1075b cm^{-1}; ^1H NMR δ5.40 (2H, *m*, HC=CH), 3.63 (3H, *m*, CH_2OH, R_2CHOSi), 2.18 and 2.00 (4H, *m's*, CH_2CH=$CHCH_2$), 1.56 (2H, *m*, CH_2CH_2O), 1.25 (20.8H, CH_2s), 0.88 (12H, *t*Bu, RCH_3), 0.039 (6H, *s*, $SiCH_3$) ppm; ^{13}C NMR δ131.37 and 125.94 (C=C), 72.42 (R_2CHOSi), 63.04 (CH_2OH), 25.91 (*t*Bu), –4.36 ($SiCH_3$) ppm. The monosilylated diol (10.2 g, 25.8 mmol) and triethylamine (4.1 mL, 29.4 mmol) were stirred in THF (80 mL, dried over KOH) protected by a drying tube and chilled to 0–5°C. A solution of methanesulfonyl chloride (2.2 mL, 28.4 mmol) in THF (20 mL) was added dropwise. The mixture was stirred at ambient temperature for 2 h, and after adding lithium bromide (13.1 g, 0.15 mol), was allowed to stir an additional 16 h. The reaction was worked up in the usual fashion with hexane and water. The organic phase was extracted sequentially with 2N HCl and water, dried ($MgSO_4$), and concentrated. The crude product was purified by flash chromatography with the same column and overloading (5% EA–H) to give **2**; 8.5 g, 71.4%: TLC 15% EA–H, $R_f = 0.78$; IR 1070b cm^{-1}; ^1H NMR δ5.40 (2H, *m*, CH=CH), 3.62 (^1H, *m*,

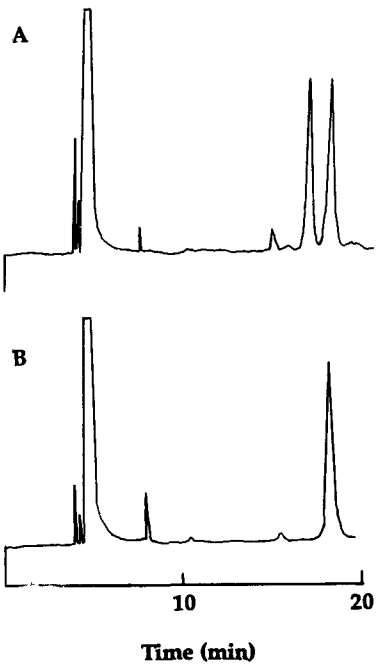


FIG. 1. Chromatograms (high-performance liquid chromatography) of the diastereomeric carbamates formed with (*S*)-1-(1′-naphthyl)ethylisocyanate using hexane/ethyl acetate/tetrahydrofuran (95:5:1), 1.0 mL min^{-1}, and a silica gel column. A: Racemic methyl lesquerolate; B: methyl lesquerolate from lesquerella oil.

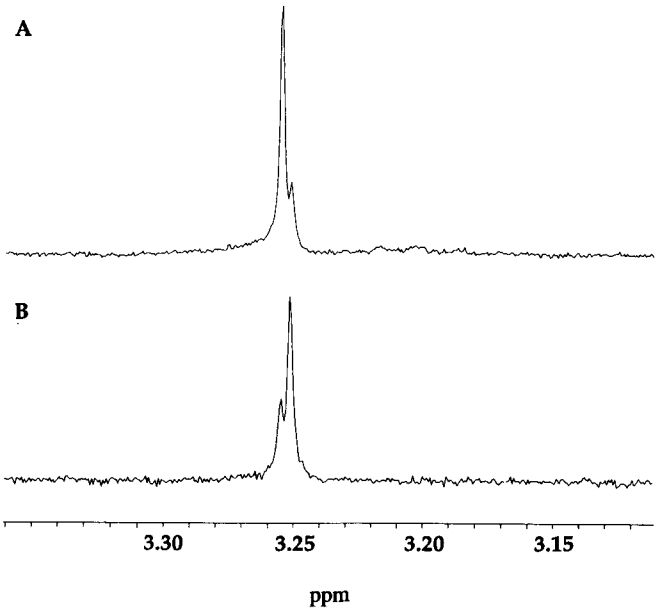
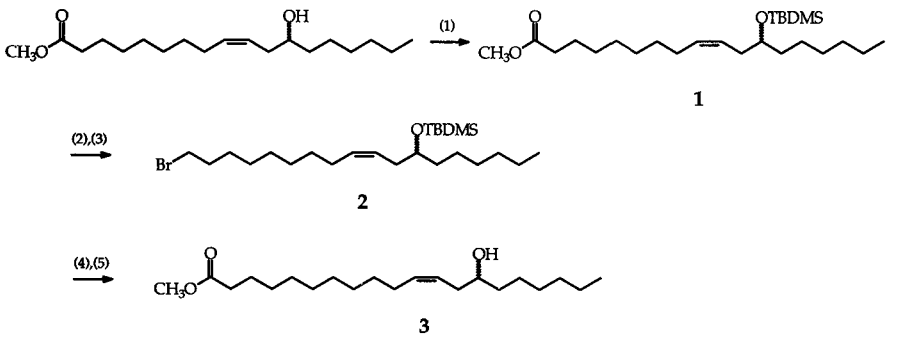


FIG. 2. ^1H nuclear magnetic resonance spectra of high-performance liquid chromatography-collected carbamates show differentiated signals for $-CO_2CH_3$. A: Early eluting *S,S*-diastereomer; B: later eluting $R_{(alcohol)}$, $S_{(amine)}$ diastereomer.



SCHEME 1

R_2CHOSi), 3.40 (2H, *bt*, CH_2Br), 2.18 and 2.00 (4H, *m's*, $CH_2CH{=}CHCH_2$), 1.93 (2H, *m*, CH_2CH_2Br), 1.28 (19.6H, CH_2s), 0.89 (12H, *t*Bu and RCH_3), 0.044 (6H, *s*, $SiCH_3$) ppm; ^{13}C NMR δ131.31 and 126.00 (C=C), 72.42 (R_2CHOSi), 25.91 (*t*Bu), -4.36 ($SiCH_3$) ppm.

Preparation of (R)*-14-hydroxy-Z-11-eicosenoic acid (lesquerolic acid), methyl ester,* **3**. *t*-Butyl acetate (2.7 mL, 20 mmol) was injected into a solution of lithium cyclohexylisopropylamide under nitrogen and at −78°C. The amide had been prepared from *n*-butyllithium (8.0 mL of 2.5 M in hexane) and the amine (3.3 mL, 20 mmol) in dry THF 40 mL). After 20 min, **2** (8.36 g, 18.2 mmol) in HMP (8 mL) was added at one time. The resulting mixture was stirred overnight at room temperature. The reaction was worked up by dilution with 1N HCl and extraction into hexane. The organic phase was washed thoroughly with water, dried ($MgSO_4$), and concentrated. Flash chromatography (5% EA–H) gave 5.45 g, 60.6% of alkylation product. The *t*-butyl ester (1.64 g, 3.61 mmol) was deblocked and transesterified by warming in methanol that contained several mg of *p*-toluenesulfonic acid at 60°C for 16 h. The ester **3** was worked up in the usual manner to give 0.84 g (64%) of **3**. A sample was purified by flash chromatography with 5% EA–H: TLC 15% EA–H, R_f = 0.31; GLC 13.7 min (methyl ricinoleate 9.3 min); ^1H NMR δ5.54, 5.38 (2H, *m's*, CH=CH), 3.62 (4H, OCH_3, R_2CHOH), 2.26 (2H, *m*, $CH_2CO_2CH_3$), 2.17, 2.01 (4H, *m's*, $CH_2CH{=}CHCH_2$), 1.65 (2H, *m*, $CH_2CH_2CO_2CH_3$), 1.29 (*ca.* 22H, CH_2s), 0.84 (3H, *bt*, RCH_3) ppm; ^{13}C NMR δ174.02 (C=0), 133.47, 133.13 (C=C), 71.66 (R_2CHOH) and 51.17 (OCH_3) ppm.

RESULTS AND DISCUSSION

A sample of lesquerolic acid (84.5% pure) was available from related work (6). Other materials present were auricolic acid, $20{:}2^{11,17}$-OH^{14} (6.0%), palmitic, and stearic acids (5.7%), and a few percentage of diglycerides (GLC). Treatment with boron trifluoride•methanol provided the methyl esters. A sample of that mixture was oxidized to the ketoester with dichromate and then reduced with methanolic sodium borohydride to the racemic lesquerolic ester. Reaction with (*S*)-1-(1′-naphthyl)ethylisocyanate produced diastereomeric carbamates that were well-separated by HPLC (Fig. 1A). Collections from HPLC provided mixtures of the ^1H NMR signals which allowed association of diastereomeric structure with elution order in complete analogy to the work with methyl ricinoleate (1) (Fig. 2) [*Note:* The legends for Figures 2 and 3 in Reference 1 are unfortunately reversed]. The later-eluting diastereomer exhibited the higher field methyl ester signal because of selective shielding by the naphthyl ring and was the $(R)_{alcohol}$-$(S)_{amine}$diastereomer.

Another sample of the methyl ester of the natural product was carbamylated directly and yielded only the later-eluting compound (Fig. 1B), indicating that the lesquerolic acid in hand had the (*R*)-configuration, as determined earlier, and was essentially configurationally pure. Because synthetic methodology has advanced greatly since the seminal work of Applewhite (4), we provided an alternate route from methyl ricinoleate to methyl lesquerolate (Scheme 1): (1) *t*-Butyldimethylsilyl chloride (TBDMS-Cl), imidazole, DMF; (2) LAH; (3) methanesulfonyl chloride, triethylamine, LiBr, THF; (4) *t*-butyl lithioacetate, THF, HMPT, (5) *p*-toluenesulfonic acid, methanol; the carbamylated product was the same as that from natural lesquerolic acid.

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