Synthesis of the Anti-Melanogenic Glycerol Fatty Acid Ester Isolated from the Tuber-Barks of *Colocasia antiquorum* var. *esculenta*

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Abstract: (2′*S*)-1-*O*-9-Oxo-(10*E*,12*E*)-octadecadienoyl glycerol, a natural anti-melanogenic monoglyceride, is synthesized for the first time. The chiral pool based route employed not only confirms the absolute configuration, but also illustrates the first synthetic entry to the (*E*,*E*)-diene keto acid, which is another molecule of biological importance. The confusion caused by the misinterpreted ^{1}H NMR spectroscopic data for the (*E*,*E*)-diene motif in the literature is discussed. The first unequivocal piece of evidence for the assigned (12*E*) configuration is also presented.

Key words: diols, acylation, glycerolipids, rearrangement, natural products

Naturally occurring monoacylglycerols [i.e., 1(or 3)-acyl-*sn*-glycerols **1**–**4**, Figure 1], often featuring a long alkyl group in the acyl subunit, and with or without additional functionalities on the acyl chain, represent an important family of lipids.[1] Such species are also essential precursors for the preparation of asymmetric di- or tri-acylglycerols. For these reasons, the development of methods to access enantiopure 1(or 3)-acyl-*sn*-glycerols with a predefined absolute configuration has received significant attention.[2]

(2′*S*)-1-*O*-9-Oxo-(10*E*,12*E*)-octadecadienoyl glycerol (**4**) is an optically active natural monoglyceride, which was recently isolated from the tuber-barks of *Colocasia antiquorum* var. *esculenta* by Lee and co-workers.[3] In preliminary testing, compound **4** showed inhibitory effects on melanin biosynthesis in melan-a cells.[3] There are also reports on the involvement of this compound and its isomers, as well as the corresponding fatty acids (e.g., **5** and **6**, two of a group of diene-keto acids that are often known as KODEs, Figure 2), in different biological processes.[4–6]

Given the significance of **4** and the fact that its structure and the configurations were established on the basis of spectroscopic analyses without any support from, for example, chemical degradations and close comparison with a known/authentic compound, it appeared desirable to us to further secure the molecular identity of **4** by a chemical synthesis.

As noted in previous studies,[2a,7] the difficulties (caused by facile acyl migrations even under mild conditions) with the synthesis and handling of 1(or 3)-acyl-*sn*-glycerols tends to be belied greatly by their unpretentious structures. In the case of **4**, the presence of a diene motif in conjugation with a ketone carbonyl group certainly introduces further unstable factors and complications compared with ester **1**, and thus makes its synthesis a worthy endeavor.

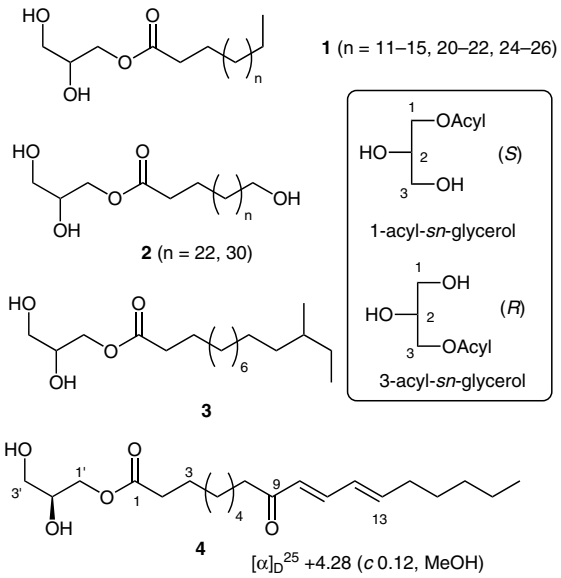

Figure 1 Some of the known naturally occurring 1(or 3)-acyl-*sn*-glycerols, with the definitions for 1- and 3-acyl-*sn*-glycerols commonly employed in lipid chemistry shown in the box. *sn* = stereospecific numbering

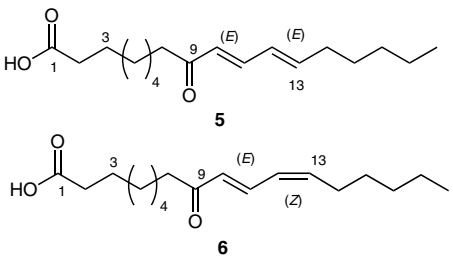

Figure 2 The structures for the (10*E*,12*E*)-diene-9-keto acid **5** related to the acyl group present in the monoglyceride **4** and its (12*Z*)-isomer **6**

Our efforts to gain access to the diene-keto acid **5** began with the route shown in Scheme 1.[8] To secure a mandatory (*E*,*E*)-diene configuration, we opted for the triphenylphosphine-mediated isomerization approach developed

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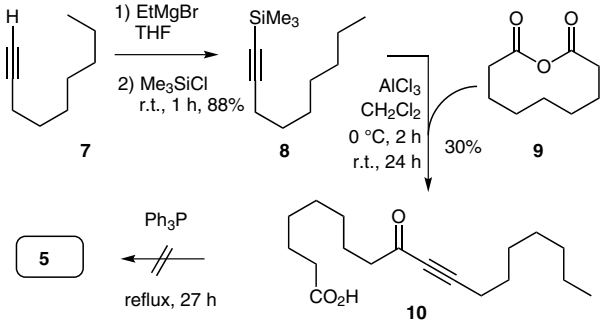
by Guo and Lu,[9] which had been shown to be effective for converting many conjugated ynones into the corresponding (*E*,*E*)-diene ketones. The desired conjugated ynone motif was assembled by catenation of commercially available 1-nonyne (**7**) with azelaic anhydride (**9**),[10] via an aluminum chloride ($AlCl_3$) mediated acylation of the corresponding alkynylsilane **8**,[11] prepared by treatment of **7** with ethylmagnesium bromide (EtMgBr) and trimethylsilyl chloride (Me_3SiCl). It is noteworthy that although there are some precedents of acylations of $Me_3SiC{\equiv}CSiMe_3$,[12] or monosilylated alkynes with the triple bond in conjugation with a phenyl ring or a heterocycle,[13] the present result appears to be the first case for the non-functionalized alkyl-substituted silylalkynes (to our knowledge).[14]

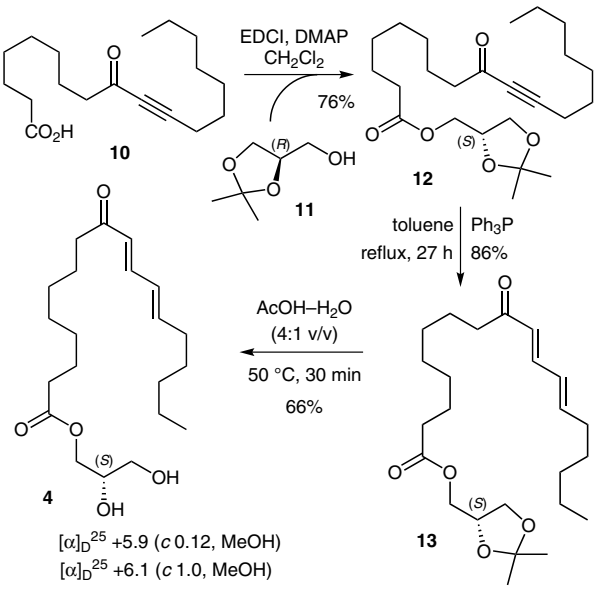


Scheme 1 An expeditious assembly of a fully functionalized backbone **10** for the synthesis of **4**

The acid **10** was next examined for the isomerization in the presence of triphenylphosphine. Unexpectedly, instead of the desired acid **5**, a mixture of unidentified side products was consistently obtained no matter what sets of conditions, as recommended in the literature, were employed. The problem was later solved by converting the acid **10** into the corresponding ester **12** via an esterification with the commercially available alcohol **11** (Scheme 2). Using ester **12** as the alkyne–diene isomerization substrate, the anticipated diene **13** formed readily, even under the mildest set of known conditions (using a catalytic amount of Ph_3P at ambient temperature). However, to ensure a satisfactory yield of **13** it was necessary to use a stoichiometric amount of triphenylphosphine and to run the reaction at reflux temperature in toluene.

The hydrolysis of the acetonide protecting group was then performed under the conditions originally developed by Mori[2a] and further studied in our previous work.[7] Thus, treatment of **13** with acetic acid–water (4:1) at 50 °C for 30 minutes[15] led to the desired product **4** in 66% isolated yield (along with 34% unhydrolyzed **13**).[16]

The synthetic **4**[17] thus obtained showed ^{13}C NMR spectroscopic data that was fully consistent with those reported[3] for the natural sample. The ^1H NMR spectroscopic data were also in good agreement with those[3] for the natural **4**, except for the H-12 and H-13 signals of the synthetic sample (Figure 3), which did not seem to fit with the descriptions for the natural product [δ 6.28 (dd, *J* = 11.5, 15.0 Hz,



Scheme 2 Conversion of acid **10** into glycerol **4**

H-12) and δ 6.27 (dd, *J* = 5.5, 15.0 Hz, H-13)],[3] even if presuming that the δ 6.27 'dd' was a typographical error for a reasonable 'dt'. Indeed, given the almost identical chemical shifts for the two mutually coupled protons, the spectrum is not likely to be of first order.

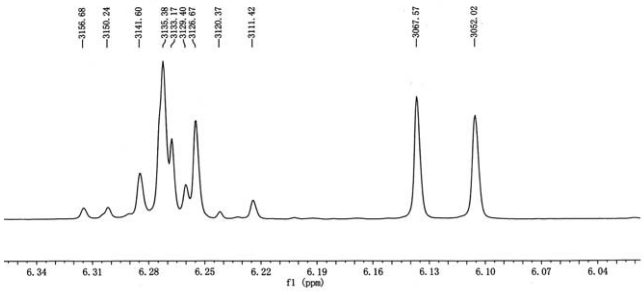


Figure 3 The ^1H NMR signals for the H-12 and H-13 protons of synthetic **4** (500 MHz, CD_3OD), with the δ 6.12 doublet (*J* = 15 Hz) for H-10 shown as a convenient scale for estimating the line-splitting in the multiplet(s). The spectrum for **4** recorded in $CDCl_3$ looked almost the same (see the Supporting Information).

The (12*E*) configuration of the natural product **4** was deduced through comparison of its ^1H NMR spectrum with that[6a] (taken in $CDCl_3$) for the corresponding free (10*E*,12*E*)-acid (**5**).[3] However, the cited data did not contain any information about the line-splitting patterns [which reads: δ 6.15 (m, 2 H, H-12 and H-13)].[6a,b] We also checked with all the records for compound **5** covered by SciFinder, and surprisingly found that despite its claimed (10*E*,12*E*) configuration in the literature, neither an explicit/reliable coupling constant (except in ref. 3), nor a scanned spectrum that might support the (12*E*) geometry for **5** (or any of its esters) had been published.

We next converted our sample of **4** into acid **5** by saponification and recorded the ^1H NMR spectrum for **5** in

$CDCl_3$ as reported in the literature,[18] with the hope to observe some evidence for the (12*E*) configuration. To our disappointment, the signals for the H-12 and H-13 protons in the ^{1}H NMR (500 MHz, $CDCl_3$) spectrum for **5** turned out very similar to that of **4** shown in Figure 3; it therefore remained impossible to assign a (12*E*) configuration.

Subsequently, we found that Lu and co-workers[19] had elegantly solved similar problems (but not related to glycerides) by recording the ^{1}H NMR spectra in benzene-d_6, in which the counterparts for the H-12 and H-13 protons of **4** (and **5**) were resolved into a 'dd' and a 'dt' signal, respectively, and thus proved the (*E*,*E*) configurations for their conjugate diene-ketones.

Using the same technique, the H-12 and H-13 protons of **5** were also resolved from each other, and the distinct signals at δ 5.96 (dd, J = 15.2, 10.8 Hz, 1 H, H-12) and δ 5.79 (dt, J = 15.1, 7.2 Hz, 1 H, H-13) allowed for an unequivocal assignment of a (12*E*) configuration for **5** (and *a priori*, **4**).[20] The long-missing piece of solid evidence for the configuration of the C-12/C-13 double bond for acid **5** and related compounds is now finally available.

In summary, the title monoglyceride **4** was synthesized through a chiral pool based route, which allowed for a definite confirmation of the structure as well as the configurations assigned previously on the basis of spectroscopic analyses/empirical rules. The present work also illustrates the only (to date) synthetic entry to (10*E*,12*E*)-9-oxo-octadeca-10,12-dienoic acid (**5**), another biologically important substance that has been involved in many lipids studies, but which does not seem to have ever been fully characterized, at least as far as the configuration of the C-12/C-13 double bond is concerned. As the first piece of solid evidence for the (12*E*) configuration for **5**, and all closely related compounds (including **4**), is finally available by recording the ^{1}H NMR spectra in benzene-d_6,[21] the long-missing (although unknown) confusion/uncertainty regarding the (12*E*) configuration of the related compounds can thus be eliminated from now on.

Acknowledgment

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Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett.

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(14) Direct treatment of **9** with the anion of alkyne **7** led to extensive formation of unidentified side products.

(15) Here we deliberately stopped the hydrolysis early to ensure that no over-reaction/acyl migrations would occur.

(16) A solution of acetonide **13** (91 mg, 0.22 mmol) in AcOH–H_2O (4:1 v/v, 2.3 mL) was stirred at 50 °C (oil bath) for 30 min. The heating bath was removed, and sat. aq $NaHCO_3$ solution (2 mL) was added followed by EtOAc (3 mL). The phases were separated and the aqueous layer was back-extracted with EtOAc (2 × 3 mL). The combined organic layers were washed with brine (3 mL) before being dried over anhydrous Na_2SO_4. Removal of the solvent on a rotary evaporator and column chromatography (PE–EtOAc, 2:1) on silica gel afforded diol **4** (53.9 mg, 66%) as a white solid along with unhydrolyzed starting **13** (31 mg, 0.075 mmol, 34%). Data for **4**: Mp 52–54 °C; $[\alpha]_D^{26}$ +5.9 (*c* 0.12, MeOH), $[\alpha]_D^{26}$ +6.1 (*c* 1.00, MeOH), $[\alpha]_D^{27}$ +11.7 (*c* 0.12, DMSO), $[\alpha]_D^{27}$ +7.0 (*c* 0.54, DMSO), $[\alpha]_D^{27}$ +7.8 (*c* 1.00, DMSO) {Lit.[3] $[\alpha]_D^{25}$ +4.28 (*c* 0.12, MeOH)}. IR (film of a concd soln in CH_2Cl_2): 3359 (br), 2928, 2852, 1732, 1682, 1634, 1596, 1471, 1406, 1376, 1332, 1310, 1236, 1223, 1182, 1118, 1045, 997 cm^{-1}. ^1H NMR (500 MHz, CD_3OD): δ = 7.23 (dd, *J* = 15.6, 9.8 Hz, 1 H), 6.33–6.21 (m, 2 H), 6.12 (d, *J* = 15.6 Hz, 1 H), 4.15 (dd, *J* = 11.4, 4.4 Hz, 1 H), 4.06 (dd, *J* = 11.4, 6.3 Hz, 1 H), 3.85–3.78 (m, 1 H), 3.56 (dd, *J* = 11.3, 5.4 Hz, 1 H), 3.53 (dd, *J* = 11.3, 5.7 Hz, 1 H), 2.60 (t, *J* = 7.4 Hz, 2 H), 2.35 (t, *J* = 7.4 Hz, 2 H), 2.20 (br q, *J* = 6.8 Hz, 2 H), 1.62 (quin, *J* = 6.9 Hz, 2 H), 1.58 (quin, *J* = 7.2 Hz, 2 H), 1.47 (quin, *J* = 7.4 Hz, 2 H), 1.39–1.30 (m, 10 H), 0.91 (t, *J* = 7.0 Hz, 3 H). ^{13}C NMR (125 MHz, CD_3OD, with the solvent multiplet set at δ 48.0 as the internal reference): δ = 202.8, 174.4, 146.3, 144.3, 129.2, 127.8, 70.1, 65.5, 63.1, 40.0, 33.9, 33.1, 31.5, 29.15, 29.14, 29.0, 28.5, 24.9, 24.5, 22.5, 13.4. ESI-MS: *m*/*z* = 369.5 $[M + H]^+$, 391.5 $[M + Na]^+$. ESI-HRMS: *m*/*z* $[M + Na]^+$ calcd for $C_{21}H_{36}O_5Na$: 391.2455; found: 391.2456.

(17) The optical rotation for synthetic **4** was measured to be $[\alpha]_D^{25}$ +5.9 (*c* 0.12, MeOH) and $[\alpha]_D^{25}$ +6.1 (*c* 1.00, MeOH); cf. the data reported (see ref. 3) for natural **4**: $[\alpha]_D^{25}$ +4.28 (*c* 0.12, MeOH).

(18) It is worth mentioning that the ^1H and ^{13}C NMR spectra of **4** recorded in $CDCl_3$ (see the Supporting Information) were very similar to those for its functionality regioisomer, (2′*S*)-1-*O*-13-oxo-(9*E*,11*E*)-octadecadienoyl glycerol,[6a] another natural monoglyceride. However, small yet definite differences also exist, representing an interesting piece of evidence for differentiating these two closely related biologically important substances.

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(20) It appears that all previous investigators working with **6** and related compounds were not aware of the advantage of using benzene-d_6. Otherwise, the lack of evidence for the (12*E*) configuration for **6** would not have gone unnoticed until now.

(21) Since the (12*E*) configuration for compounds **5** and **4** in this work have now been secured by analysis of the ^1H NMR spectra in benzene-d_6, the expansion of the ^1H NMR spectrum for the H-12 and H-13 region of acid **5** (and/or **4**), recorded in CD_3OD or $CDCl_3$ (both are much more commonly employed in synthesis than benzene-d_6), provided in this work may thus be used as a quick reference for confirming the (12*E*) configuration for closely related compounds. One of the referees pointed out that the use of decoupling techniques could also provide pertinent information about the double-bond configuration.

