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OXYGENATED FATTY ACIDS OF ISANO OIL

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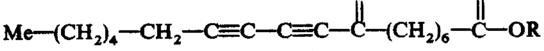
Key Word Index—*Ongokea gore*; Olacaeae; isano; seed oil; oxygenated acetylenic acids; ORD; PMR; ^{13}C NMR; MS.

Abstract—Four new oxygenated acetylenic acids—8-oxo-octadeca-9,11-diynoic (**1a**), 8-hydroxyoctadeca-10,12-diynoic (**3a**), 8-oxo-octadeca-9,11-diyn-17-enoic (**4a**) and 8-hydroxyoctadeca-10,12-diyn-17-enoic (**6a**) acids—and a saturated acid, 9L-hydroxyoctadecanoic acid (**7a**), have been isolated for the first time from the seed oil of *Ongokea gore* (isano). Also isolated were the previously known 8-hydroxyoctadeca-9,11-diynoic (**2a**) and 8-hydroxyoctadeca-9,11-diyn-17-enoic (**5a**) (isanolic) acids. Their structures have been determined by PMR and ^{13}C NMR and MS, and the absolute configurations of **5a** and **7a** have been established by ORD.

INTRODUCTION

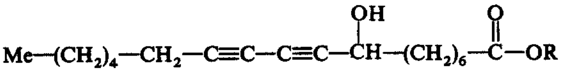
The seed oil of *Ongokea gore* Engler (isano) has been of both academic and commercial interest because of the acetylenic fatty acids present in its triglycerides [1–8]. The extensive literature on this unusual oil up to 1963 has been thoroughly reviewed by von Mikusch [1]. The occurrence in isano oil of 9 different acetylenic acids, 4 of which have OH substituents, appeared to be well established. The most definitive characterization work on these acetylenic acids was published in 1963 by Gunstone *et al.* [6] and by Morris [7]. Along with numerous previous investigators, they reported the occurrence of acids with a conjugated *cis*-enediyne UV chromophore, but they did not succeed in isolating pure compounds responsible for this absorption. We undertook a new investigation of this oil in the hope of obtaining these elusive enediynes in a pure form by application of countercurrent distribution in conjunction with other separation techniques. We isolated and characterized 5 new oxygenated acids, two of which account for a portion of the 'enediyne' absorption of isano oil through their unexpected diynone structures. However, much of this absorption remains unaccounted for structurally.

RESULTS AND DISCUSSION



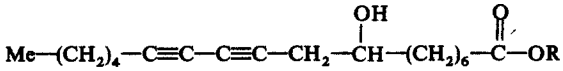
$$\mathrm{Me{-}(CH_2)_4{-}CH_2{-}C{\equiv}C{-}C{\equiv}C{-}\overset{O}{\overset{\|}{C}}{-}(CH_2)_6{-}\overset{O}{\overset{\|}{C}}{-}OR}$$

(**1a**) R = H
(**1b**) R = Me



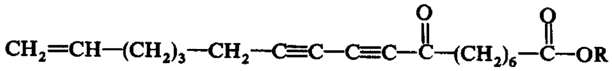
$$\mathrm{Me{-}(CH_2)_4{-}CH_2{-}C{\equiv}C{-}C{\equiv}C{-}\overset{OH}{\overset{|}{C}H}{-}(CH_2)_6{-}\overset{O}{\overset{\|}{C}}{-}OR}$$

(**2a**) R = H
(**2b**) R = Me



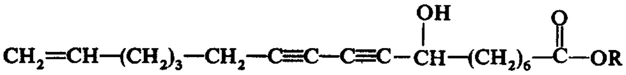
$$\mathrm{Me{-}(CH_2)_4{-}C{\equiv}C{-}C{\equiv}C{-}CH_2{-}\overset{OH}{\overset{|}{C}H}{-}(CH_2)_6{-}\overset{O}{\overset{\|}{C}}{-}OR}$$

(**3a**) R = H
(**3b**) R = Me



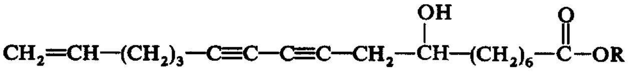
$$\mathrm{CH_2{=}CH{-}(CH_2)_3{-}CH_2{-}C{\equiv}C{-}C{\equiv}C{-}\overset{O}{\overset{\|}{C}}{-}(CH_2)_6{-}\overset{O}{\overset{\|}{C}}{-}OR}$$

(**4a**) R = H
(**4b**) R = Me



$$\mathrm{CH_2{=}CH{-}(CH_2)_3{-}CH_2{-}C{\equiv}C{-}C{\equiv}C{-}\overset{OH}{\overset{|}{C}H}{-}(CH_2)_6{-}\overset{O}{\overset{\|}{C}}{-}OR}$$

(**5a**) R = H
(**5b**) R = Me



$$\mathrm{CH_2{=}CH{-}(CH_2)_3{-}C{\equiv}C{-}C{\equiv}C{-}CH_2{-}\overset{OH}{\overset{|}{C}H}{-}(CH_2)_6{-}\overset{O}{\overset{\|}{C}}{-}OR}$$

(**6a**) R = H
(**6b**) R = Me

Separation

The column separation of the isano fatty acids (FA) yielded 66% nonpolar acids, 29% oxygenated acids and 5% of very polar material. The results on the nonpolar acids will be reported later. The countercurrent (CCD) fractionation of the hydroxy fatty acid Me esters (FAME) was not as effective as had been anticipated. After 1500 transfers, CCD fraction B (see Experimental) was not completely separated from fraction C; furthermore, each of these 2 fractions was composed of 3 esters. Argentation TLC was used to monitor the fractionation from CCD. Fractions B and C each gave essentially one spot on $AgNO_3$–TLC (R_f 0.65 and 0.48, respectively), but when these fractions were examined on Si gel, 3 spots from each were noticed: R_f 0.69 (**1b**), 0.44 (**2b**) and 0.28 (**3b**) for B; R_f 0.67 (**4b**), 0.40 (**5b**) and 0.26 (**6b**) for C. These components of the two fractions were separated by PLC.

Characterization of the diynols

The major hydroxy FAME, **2b** and **5b**, were characterized by previous workers [1, 2, 6, 7]. The IR of both indicated OH (3610–3620 cm^{-1}), one medium intensity C≡C stretch band (2260–2270 cm^{-1}) and a strong ester

Table 1. PMR of the oxygenated methyl diynoates from isano oil. (Chemical shifts, δ, were measured in ppm from TMS in $CDCl_3$ solutions; J are expressed in Hz)

Carbon	**1b**	**4b**	**2b**	**5b**	**3b**	**6b**
OMe	3.62 *s*	3.62 *s*	3.62 *s*	3.62 *s*	3.62 *s*	3.63 *s*
2	2.27 *t* $J = 7$	2.28 *t* $J = 7$	2.28 *t* $J = 7$	2.28 *t* $J = 7$	2.28 *t* $J = 7$	2.28 *t* $J = 7$
3–6	1.2–1.8 *m*	1.2–1.8 *m*	1.2–1.8 *m*	1.2–1.8 *m*	1.2–1.8 *m*	1.2–1.8 *m*
7	2.52 *t* $J = 6.5$	2.52 *t* $J = 7$	1.6 *m*	1.6 *m*		
8			4.34 *t* $J = 6$	4.34 *t* $J = 6$	3.70 *m*	3.71 *m*
9					2.40 *d* $J = 6$	2.41 *d* $J = 6$
13	2.33 *t* $J = 7$	2.35 *t* $J = 7$	2.24 *t* $J = 6$	2.25 *t* $J = 7$		
14	1.2–1.8 *m*	1.2–1.8 *m*	1.2–1.8 *m*	1.2–1.8 *m*	2.20 *t* $J = 7$	2.25 *t* $J = 7$
15					1.2–1.8 *m*	1.6 *tt* $J = 7,8$
16		2.06 *m*		2.04 *m*		2.1 *m*
17		5.73 *m*		5.74 *m*		5.74 *m*
18	0.89 *t* $J = 6$	4.97 *m*	0.87 *t* $J = 6$	4.96 *m*	0.88 *t* $J = 6$	5.0 *m*

carbonyl (1730 cm^{-1}). The vinyl group of **5b** was indicated by IR (3080, 1635, 1430 and 910 cm^{-1}) and PMR (multiplets at δ 4.96 and 5.74). PMR showed the methylene protons next to the triple bonds as two-proton triplets ($J = 6$ and 7 Hz) at δ 2.24 and 2.25, and the proton at C-8 as one-proton triplets ($J = 6$ Hz) at δ 4.34 (Table 1) similar to the signal for the corresponding grouping in heptadec-16-ene-4,6-diyn-8-ol [9].

MS indicated that the OH group was on C-8 in both **2b** and **5b**. An ion at *m/e* 144, formed by 7,8-cleavage with abstraction of one H from the fragment eliminated [10a] is observed from both compounds. Ions at *m/e* 163 (**2b**) and 161 (**5b**) also arise from 7,8-cleavage in which the unsaturated fragment receives the charge. The absence of an intense ion from 8,9-cleavage indicates conjugation of the OH group on C-8 with unsaturation at C-9. Low-intensity ions are observed at *m/e* 221 arising from 12,13-cleavage, and at *m/e* 236 from 13,14-cleavage accompanied by a rearrangement in which a H migrates from C-15 to C-11 [10b]. The conjugated diynol system was further indicated by *m/e* 91, arising from either $H_2C{=}C{=}CH{-}C{\equiv}C{-}CO$ or $C{\equiv}C{-}C{\equiv}C{-}C(OH){-}CH_2$ [10b]. MS of the silylated derivatives supports the same conclusions.

ORD of methyl 8-hydroxyoctadecanoate obtained from hydrogenation of **5b** established that C-8 has the D configuration, since it is a plain negative curve in MeOH solution [11]. There was 3.3% of **2a** and 12.5% of **5a** in the original isano oil.

In addition to diynols **2b** and **5b**, two previously unrecognized isomers of these diynols, **3b** and **6b**, were isolated by PLC of CCD fractions B and C, respectively (*vide supra*). Initially, fractions B and C appeared fairly homogeneous as judged by GLC and argentation TLC. The first hint of the occurrence of **3b** and **6b** was given by a ^{13}C NMR spectrum of fraction C. Signals due to both double-bonded carbons (C-17 and C-18) were accompanied by satellite peaks differing in chemical shift from the major peaks by about 1 ppm (see **5b** and **6b** in Table 2). Diynols **3b** and **6b** had distinctly different spectral properties from **2b** and **5b**, including plain ORD curves with opposite signs of rotation.

MS demonstrated that **3b** and **6b** were isomeric with, although different from, **2b** and **5b**. MS of **3b** and **6b** located the OH group at C-8 and demonstrated the absence of unsaturation between C-1 and C-8. However, in contrast to **2b** and **5b**, the spectra of **3b** and **6b** show intense ions at *m/e* 173 (245 in TMSi derivative) arising from 8,9-cleavage instead of ions indicating 7,8-cleavage. Accordingly, at least one methylene group must occur between C-8 and the triple bond system. PMR spectra clearly demonstrated the absence of double bonds (apart from the *terminal* double bond in **6b**) and also indicated that **3b** and **6b** have no acetylenic linkage adjacent to the OH-bearing C-8, since the methine proton attached to this carbon appeared considerably upfield in **3b** and **6b** (δ 3.70) compared to its position in **2b** and **5b** (δ 4.34) [12a]. The absence of acetylenic

Table 2. ^{13}C NMR assignments for methyl hydroxydiynoates from isano oil (chemical shifts, δ, were measured in ppm from TMS in $CDCl_3$ solution)

Carbon	**2b**	**3b**	**5b**	**6b**
OMe	51.4	51.4	51.4	51.5
1	174.3	174.2	174.4	174.2
2	34.1	34.1	34.1	34.1
3	24.9	24.9	24.9	24.9
4	28.9	29.1	29.1	29.1
5	28.9	29.1	28.9	29.1
6	24.9	28.4	24.9	28.3
7	37.6	36.4	37.6	36.4
8	62.8	70.1	62.8	70.1
9	81.7*	25.4	81.4*	25.4
10	70.0*	77.9*	69.9*	77.9*
11	64.5*	67.8*	64.7*	67.8*
12	76.7*	65.1*	76.8*	65.4*
13	19.3	73.5*	19.2	73.6*
14	28.5*	19.2	28.0	18.6
15	28.2*	28.0	27.6	27.6
16	31.3	31.1	33.1	32.8
17	22.5	22.1	138.4	137.5
18	14.0	13.9	114.8	115.5

* Doubtful assignments.

absorption near 2260 cm^{-1} in the IR of **3b** and **6b** suggested that their two triple bonds were not conjugated; however, there is precedent for the absence of such IR bands in unsymmetrically substituted conjugated diynes [13].

A methylene-interrupted arrangement of the triple bonds in **3b** and **6b** was rejected because they showed no PMR signal near δ 2.95 as observed in synthetic methylene-interrupted diynes [12]. MS of products from methoxymercuration of **3b** established O labeling at C-11 and C-13 and suggested that **3b** was either a 10,12- or an 11,13-diyne. The 10,12-diyne structure was supported by the PMR spectra of **3b** and **6b** (Table 1) which contained a two-proton doublet ($J = 6$ Hz) at δ 2.4 attributable to C-9 [14] which collapsed to a singlet when δ 3.7 (signal for C-8) was irradiated. The signal for C-14 is a two proton triplet ($J = 7$ Hz) at δ 2.20 for **3b** [15] and is farther downfield for **6b** (at δ 2.25) because of the vinyl group. The signal for C-15 of **6b** is an apparent quintet centered at δ 1.6 (compare with compounds having 3 methylene groups between two triple bonds in [12b]).

The ^{13}C NMR of **3b** and **6b** indicated acetylenic carbon signals in the same region as for **2b** and **5b**, (see Table 2); however, the shift for C-8 in **3b** and **6b** (δ 70.1) is considerably downfield from that of **2b** and **5b** (δ 62.8) and approaching that of saturated hydroxy esters [16]. We attribute this difference to the shielding effect of the triple bonds; the insertion of a methylene group (C-9) attenuates this effect considerably [17]. Isano oil contained 0.7 % **3a** and 3.7 % **6a**.

Characterization of the diynones

IR spectra gave the first clue as to the identity of **1b** and **4b**. Very strong C≡C stretch absorption was noted at 2230 and 2160 cm^{-1} along with two strong carbonyl bands at 1730 and 1665 cm^{-1}; OH absorption was absent. The very intense acetylenic absorption bands were unexpected, since they are usually weak or absent unless four or more triple bonds in conjugation are present [8, 18]. However, both the acetylenic and carbonyl absorption for **1b** and **4b** resemble those illustrated by Bohlmann and Kleine [13] for a conjugated diynone. Furthermore, the observed UV spectrum was in good agreement with that established for conjugated diynones [19]. The latter have UV maxima at wavelengths similar to those of conjugated enediynes [20] but their intensities are lower.

In the MS of **1b**, ions at *m/e* 161 and 171, arising from α-cleavage at either side of the carbonyl function, indicate that the carbonyl group was located at C-8. An ion at *m/e* 176 arises from 6,7-cleavage through a McLafferty rearrangement [10a]. The absence of a rearrangement ion at *m/e* 186, from 9,10-cleavage, indicates unsaturation between C-9 and an adjacent carbon. Ester **4b** gives the same ions indicating 6,7- and 7,8-cleavage as does **1b**, but lowered by two amu to *m/e* 159 and 174. Again, the rearrangement ion from 9,10-cleavage is not observed.

PMR (Table 1) confirmed the structures of **1b** and **4b**. The methylene group next to the carbonyl gives a two-proton triplet ($J = 6.5$ to 7 Hz) at δ 2.52 [9] and the methylene next to the triple bond system gives a two-proton triplet ($J = 7$ Hz) at δ 2.33 or 2.35 [9]. The vinyl group of **4b** was, of course, revealed by both IR and PMR. A question may arise as to the natural occurrence of these two diynones. They may be artifacts resulting from oxidation of conjugated diynols, although their apparent concentration is quite low (0.4 % of the original oil for **1a** and 1.2 % in the case of **4a**).

9L-Hydroxyoctadecanoic acid

Removal of solvent from fraction A left a solid whose mp and R_f matched those of authentic methyl 9L-hydroxyoctadecanoate. MS confirmed the location of the OH at C-9 rather than C-8, as might have been anticipated, since the base peak was at *m/e* 155 [10a]. MS of the silylated ester gave ions at *m/e* 229 and 259, typical cleavage products from Me esters of silylated hydroxy acids [21]. ORD of the hydroxy ester gave a plain positive curve establishing the structure as Me 9L-hydroxyoctadecanoate [11]. The isolation of a saturated hydroxy acid as a minor constitituent (1.2 %) of isano oil was surprising in itself, but even more so was the discovery that this is a 9L-hydroxy acid. Its MS was readily distinguishable from 8D-hydroxyoctadecanoic acid obtained from hydrogenation of **5b** and its ORD curve was opposite in sign.

GLC and UV absorption

Previous investigators have observed decomposition of hydroxyacetylenic FAME of isano during GLC [22]. However, by using solid supports that had been silanized and on-column injection at low temperature, we obtained chromatograms without noticeable decomposition on either nonpolar or polar packings.

In every fraction examined, there was a series of UV absorption maxima at or near 214, 228, 240, 254, 268 and 283 nm, attributed by previous workers [1, 6, 7, 8] to a *cis*-enediyne chromophore occurring in isano oil. This assignment was based on the UV spectral characteristics of octadec-*cis*-13-en-9,11-diynoic acid synthesized by Crombie and Williams [20]. Although Meade [8] reported the isolation from isano of a nonoxygenated acid (bolekic acid) with the same structure as Crombie's synthetic acid, this identification rested largely on UV spectral data [23]. We have noted that the same UV maxima could be attributed to conjugated diynones [19]. This ubiquitous UV absorption, although partially accounted for by our discovery of two diynone acids, remains unresolved since these diynones could not be present in appreciable amounts in most of the TLC fractions that show this absorption. Crombie noted a similar UV absorption in the maleic anhydride adduct of anacyclin and was unable to eliminate it by repeated recrystallization [24].

EXPERIMENTAL

General procedures. IR spectra were measured in CCl_4, UV in MeOH and PMR (100 MHz) in $CDCl_3$ with TMS as an internal standard. The ^{13}C NMR spectra were determined on a Fourier Transform NMR spectrometer, operating at 22.63 MHz with proton noise decoupling. The computer data memory size used for the real part of the spectra was 4 k. The δ values are accurate to within ± 0.1. A 5 µsec (*ca* 30°) pulse width was used. The spectra (5000–50000 accumulations) were obtained from solns in $CDCl_3$ which also served as an internal deuterium lock. Chemical shifts are given as δ-values in ppm downfield from the internal TMS-^{13}C signal. MS were measured at 70 eV (probe and GC–MS [21]). FFA were analyzed by TLC on H_3BO_3–Si gel 60 F-254 (1:8) developed in hexane–Et_2O (3:2), visualized with I_2 and/or spraying with H_2SO_4–$K_2Cr_2O_7$ and heating. FAME were analyzed by TLC on $AgNO_3$–Si gel (1:8) developed in CH_2Cl_2–Et_2O (9:1), visualized under long (366 nm) UV light after spraying with 2′,7′-dichlorofluorescein and/or charring with H_2SO_4–$K_2Cr_2O_7$.

GLC. FAME were analyzed on 122 × 0.3 cm glass column packed with 5% Apiezon L on AW-DMCS Chromosorb W and 366 × 0.3 cm glass column packed with 7% LAC-2-R-446 on AW-DMCS Chromosorb W. Analyses were carried out isothermally at 190°, with He as carrier at 40 ml/min, no injection heater and dual FID at 250°. Hydroxy FAME were analyzed on 105 × 0.3 cm stainless-steel column packed with 3% Silar 5 CP on Gaschrom Q, temp. programmed 130° to 250° at 4°/min, He as carrier at 40 ml/min, injection heater at 230° and FID at 350°.

Material. Isano oil, purchased from Pacific Vegetable Oil Corporation, was stored at −18° until examined.

Hydrolysis of isano oil. Isano oil (19.2 g) was refluxed 1.5 hr under N_2 with N KOH in 500 ml 95% EtOH [25]. Unsaponifiables (0.79 g) were extracted with Et_2O–hexane (1:1). Soaps were acidified (pH 2) with 6 N HCl and extracted with Et_2O–hexane (1:1) to yield FFA (16.7 g).

Separation procedure. FFA (6 × 2.5 g), dissolved in MeOH–C_6H_6 (2:98), were chromatographed on Si gel (100 g) using MeOH–C_6H_6 (1:49) and (1:4) [26]; 50 ml aliquots were monitored by TLC on borated Si gel. Effluents from the column were combined into 4 fractions: nonhydroxy FA (9.9 g), mixed FA (0.5 g), hydroxy FA (4.3 g) and polar substances (0.9 g). Fractions were stored at 0° until analyzed. Hydroxy FA were esterified with 2% HCl in MeOH by refluxing 2 hr under N_2. Hydroxy FAME (3.6 g) were subjected to CCD in a 200-tube Craig-Post apparatus with CH_3CN–hexane [27]. Upper phase (40 ml) and lower phase (40 ml) were used throughout the distribution. Beginning with the 200th transfer, the upper phase was collected, one transfer per tube, until 1000 transfers had occurred. The apparatus was then converted to recycle the upper phase and 500 more transfers were made. Transfers were combined on the basis of IR, $AgNO_3$–TLC, GLC and wt distribution results. Among the combined fractions were A (transfers 440–509, 0.13 g), B (CCD fundamental series tubes 111–159, 0.52 g) and C (CCD fundamental series tubes 50–110, 2.10 g).

Characterization of A. Removal of solvent from A left Me 9L-hydroxystearate, mp 49–51° (lit. [28]), mp 52–53°). ORD: $[\alpha]_D^{26}$ +0.36°, $[\alpha]_{480}^{26}$ +0.42, $[\alpha]_{360}^{26}$ +0.54° (MeOH, *c* 1.34). GLC: ECL 19.4 (ApL), 24.7 (R-446), 24.2 (Silar 5CP); $AgNO_3$–TLC: R_f 0.62. IR: ν_{max} cm^{-1} 3660 (OH), 1730 (COOMe); PMR: δ 0.86 (3H, *t*, J = 6 Hz, C-18), 1.2–1.6 (28H, *m*, C-3 to C-8, C-10 to C-17), 2.27 (2H, *t*, J = 7 Hz, C-2), 3.56 (1H, *bs*, C-9), 3.62 (3H, *s*, COOMe); MS, *m/e* (rel. int.): 296 (M^+-H_2O, 0.6), 283 (M^+-OMe, 1), 187 (25), 158 (33), 155 (100) [10a]. Silylated A with BSTFA [29], MS, *m/e* (rel. int.): 371 (M^+-CH_3, 3), 355 (M^+-OMe, 3), 259 (100), 230 (25), 229 (65). (Found: C, 72.3; H, 12.0. Calc. for $C_{19}H_{38}O_3$: C, 72.6; H, 12.2%).

Separation of B. GLC: ECL 20.6 (ApL), 29.6 (Silar 5CP); TLC: R_f 0.69, 0.44, 0.28 [n-C_6H_{14}–Et_2O (6:4)]. PLC on two plates of 2-mm layer of Si gel 60 F-254 was used to separate B (143 mg per plate). The plates were developed 5× in hexane–Et_2O (3:2): first to a height of 7.5 cm; second, to 10 cm; third, to 12.5 cm; and fourth and fifth, to 15 cm (plates were removed from chamber and dried between developments). The bands were detected with short (254 nm) UV light, and extracted from the developed plates with Et_2O to obtain 18 mg R_f 0.85–0.93 (**1b**), 167 mg R_f 0.60–0.75 (**2b**) and 37 mg R_f 0.43–0.55 (**3b**).

Methyl 8-oxo-octadeca-9,11-diynoate (**1b**). R_f 0.69 component from B was a syrup. IR: ν_{max} cm^{-1} 2230 (*vs*, $C\equiv C$), 2160 (*s*, $C\equiv C$), 1730 (*vs*, COOMe), 1665 (*vs*, C=O); UV: λ_{max} nm (log ε) 232 (3.47), 242.5 (3.57), 256 (3.77), 2.70 (3.85), 285.5 (3.75); PMR: see Table 1; MS: *m/e* (rel. int.) 304 (M^+, 2), 289 (M^+-Me, 0.5), 273 (M^+-OMe, 4), 245 (M^+-COOMe, 2), 234 (3), 219 (6), 176 (20), 171 (18), 162 (20), 161 (100), 147 (37), 133 (45), 119 (28), 105 (54), 91 (68).

Methyl 8-hydroxyoctadeca-9,11-diynoate (**2b**). Component at R_f 0.44 from B was a syrup. IR: ν_{max} cm^{-1} 3620 (OH), 2270 (*m*, $C\equiv C$), 1730 (COOMe); PMR: see Table 1; ^{13}C NMR data in Table 2; MS: *m/e* (rel. int.) 306 (M^+, 0.3), 291 (M^+-Me, 0.2), 275 (M^+-OMe, 0.3), 247 (M^+-COOMe, 0.2), 236 (7), 221 (8), 171 (33), 163 (23), 144 (39), 135 (25), 131 (24), 129 (22), 121 (33), 119 (21), 117 (34), 108 (22), 107 (43), 105 (41), 101 (44), 95 (39), 94 (20), 93 (37), 92 (21), 91 (59), 87 (100). Silylated **2b** with BSTFA [29], MS: *m/e* (rel. int.) 378 (M^+, 0.6), 363 (M^+-Me, 4), 347 (M^+-OMe, 0.9), 308 (10), 236 (26), 235 (100).

Methyl 8-hydroxyoctadeca-10,12-diynoate (**3b**). R_f 0.28 constituent from B was a syrup. IR: ν_{max} cm^{-1} 3600 (OH), 1730 (COOMe); PMR: see Table 1, ^{13}C NMR data in Table 2; MS: *m/e* (rel. int.) 306 (M^+, 0.2), 291 (M^+-Me, 0.03), 275 (M^+-OMe, 2), 247 (M^+-COOMe, 0.1) 173 (53), 141 (100), 95 (49), 91 (20). Silylated **3b** with BSTFA [29], MS: *m/e* (rel. int.) 378 (M^+, 0.2), 363 (M^+-Me, 3), 347 (M^+-OMe, 3), 246 (22), 245 (100), 235 (0.6), 141 (16). Modified lit. procedures [30] for oxymercuration-demercuration were used: 10 mg **3b** were dissolved in 5 ml MeOH, 50 mg Hg $(OAc)_2$ added, stirred to dissolve and allowed to react 60 hr at room temp. Excess $NaBH_4$ was added, after 30 min 5 ml satd aq. NaCl was added and the mixture was extracted with 5 ml × 3 hexane–Et_2O (1:1). Combined extracts were dried over Na_2SO_4 for 1 hr, filtered into weighed flask and solvent was removed *in vacuo*. Resultant products were silylated with Py–HMDS–TMCS (1:2:1) [29]. MS: *m/e* (rel. int.) 401 (0.7), 329 (7), 285 (25), 245 (26), 199 (1), 174 (12), 173 (100), 147 (23).

Separation of C. GLC ECL 20.8 (ApL), 30.4 (Silar 5CP). TLC: R_f 0.67, 0.40, 0.26 [hexane–Et_2O (3:2)]. PLC on two plates of 2-mm layer Si gel 60 F-254 was used to separate C (141 mg on first plate; 139 mg on second plate). After multiple development in hexane–Et_2O (3:2), the bands were detected as described under B above. The developed plates were extracted with Et_2O to obtain 15 mg R_f 0.86–0.92 (**4b**) 163 mg R_f 0.60–0.72 (**5b**) and 48 mg R_f 0.35–0.52 (**6b**).

Methyl 8-oxo-octadeca-9,11-diyn-17-enoate (**4b**). The R_f 0.67 component from C was a syrup. IR: ν_{max} cm^{-1} 3080, 1635, 1430 and 910 ($RCH=CH_2$), 2230 (*vs* $C\equiv C$), 2150 (*s* $C\equiv C$), 1725 (*vs* COOMe), 1665 (*vs* C=O); UV: λ_{max} nm (log ε) 209 (3.95), 230 (3.39), 241 (3.50), 255 (3.68), 268.5 (3.82), 284 (3.72); PMR: see Table 1; MS: *m/e* (rel. int.) 302 (M^+, 0.5), 287 (M^+-Me, 2), 271 (M^+-OMe, 2), 243 (M^+-COOMe, 0.3), 174 (10), 171 (26), 160 (16), 159 (58), 145 (20), 132 (25), 131 (48), 129 (20), 117 (44), 116 (34), 115 (23), 105 (26), 104 (22), 103 (22), 93 (24), 92 (17), 91 (100).

Methyl 8-hydroxyoctadeca-9,11-diyn-17-enoate (**5b**). Constituent at R_f 0.40 from C was a syrup. ORD: $[\alpha]^{26}_{D}$ +5.14°, $[\alpha]^{26}_{520}$ +6.81°, $[\alpha]^{26}_{440}$ +10.5°, $[\alpha]^{26}_{360}$ +20.1° (MeOH, *c* 2.88); IR: ν_{max} cm^{-1} 3610 (OH), 3080, 1635, 1430 and 910 (RCH=CH$_2$), 2260 (*m*, C≡C), 1730 (COOMe); PMR: see Table 1; ^{13}C NMR: see Table 2; MS: *m/e* (rel. int.) 303 (M$^+$-H, 0.6), 289 (M$^+$-Me, 1), 273 (M$^+$-OMe, 0.2), 245 (M$^+$-COOMe, 0.6), 236 (1), 221 (2), 185 (14), 175 (10), 171 (29), 161 (49), 157 (26), 145 (28) 144 (26), 143 (34), 133 (41), 132 (21), 131 (45), 129 (42), 119 (29), 117 (47), 115 (24), 107 (28), 105 (59), 101 (34), 95 (27), 93 (33), 92 (21), 91 (92), 87 (91), 83 (24), 81 (34), 79 (44), 77 (28), 74 (21), 69 (37), 67 (48), 59 (36), 55 (100). Silylated **5b** with BSTFA [29], MS: *m/e* (rel. int.) 376 (M$^+$, 0.9), 361 (M$^+$-Me, 6), 345 (M$^+$-OMe), 0·7), 308 (4), 247 (5), 245 (6), 234 (22), 233 (84), 143 (6), 131 (5), 73 (100). (Found: C, 74.6; H, 9.4. Calc. for $C_{19}H_{28}O_3$: C, 75.0; H, 9.3%).

Hydrogenation of **5b**. **5b** (0.158 g) was hydrogenated at ambient temp. and atm. pres. in 20 ml HOAc with 10% Pd–C [31]. The products were separated by PLC on 2-mm thick Si gel with hexane–Et$_2$O (7:3) and detected with 2′,7′-dichlorofluorescein. Applied 60 mg **5 b** on one plate, 100 mg on second plate and extracted the developed plates with Et$_2$O to obtain 20 mg R_f 0.83 (methyl stearate), 26 mg R_f 0.62 (methyl 8-oxostearate) and 99 mg R_f 0.25 (methyl 8-hydroxystearate), mp 52–53°, ORD: $[\alpha]^{26}_{D}$ −0.52°, $[\alpha]^{26}_{480}$ −0.86°, $[\alpha]^{26}_{360}$ −1.57°, $[\alpha]^{26}_{280}$ −2.80° (MeOH, *c* 1.07), {lit. [32] $[\alpha]^{25}_{D}$ −0.4° (MeOH, *c* 12.7)}, silylated with BSTFA [29], MS: *m/e* (rel. int.) 371 (M$^+$-Me, 3), 355 (M$^+$-OMe, 1), 246 (20), 245 (100), 243 (59). (Found: C, 72.8; H, 12.3. Calc. for $C_{19}H_{38}O_3$: C, 72.6; H, 12.2%).

Methyl 8-hydroxyoctadeca-10,12-diyn-17-enoate (**6b**). The R_f 0.26 component from C was a syrup. ORD: $[\alpha]^{26}_{D}$ −5.12°, $[\alpha]^{26}_{520}$ −8·54°, $[\alpha]^{26}_{440}$ −13.2°, $[\alpha]^{26}_{360}$ −20.3° (MeOH, *c* 0.820); IR: ν_{max} cm^{-1} 3590 (OH), 3080, 1635, 1430 and 910 (RCH=CH$_2$), 1725 (COOMe); PMR: see Table 1; ^{13}C NMR: see Table 2; MS: *m/e* (rel. int.) 304 (M$^+$, 0.2), 273 (M$^+$-OMe, 0.5), 173 (33), 141 (100), 132 (27), 117 (45), 95 (63), 91 (25). Silylated **6b** with BSTFA [29], MS: *m/e* (rel. int.) 376 (M$^+$, 0.1), 361 (M$^+$-Me, 2), 345 (M$^+$-OMe, 1), 246 (19), 245 (100), 233 (1), 141 (16).

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