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FATTY ACID COMPOSITION IN *LEMNA MINOR*—CHARACTERIZATION OF A NOVEL HYDROXY C_{16} ACID

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Key Word Index—*Lemna minor*; Lemnaceae; fatty acid composition; triacylglycerols; (10*R*)-hydroxyhexadeca-7*Z*,11*E*,13*Z*-trienoic acid.

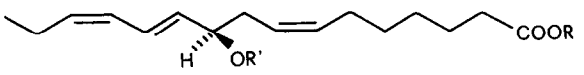
Abstract—The triacylglycerol fraction of *Lemna minor* afforded mainly 16:1 and 16:3 fatty acids and a novel hydroxy acid (10 *R*)-hydroxyhexadeca-7*Z*,11*E*,13*Z*-trienoic. The free fatty acid fraction was composed of a similarly distributed mixture.

INTRODUCTION

Lemna minor is an aquatic plant widely distributed in temperate zones. In this species adaptation to the aquatic environment causes a reduction and a simplification of the floral organs as well of the vegetative body. From a chemical point of view *L. minor* proved to be an abundant source of free fatty acids and triacylglycerols and led to the isolation of a novel unsaturated hydroxy fatty acid **1a**.

RESULTS AND DISCUSSION

L. minor, collected in the Botanical Garden of the University of Naples and identified by G. Aliotta of the University of Naples was air dried and then extracted with cold diethyl ether. Chromatography on Si gel columns of the crude extract gave three main fractions (TLC). Fraction A was composed of a complex mixture of non polar hydrocarbons (NMR, IR) which was not further investigated. Fraction B consisted of a mixture of triacylglycerols which after treatment with sodium methoxide in methanol gave mainly methyl esters of fatty acids 16:1, 16:3 and **1a**.



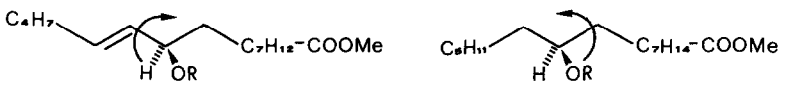
1a R = H R' = H
1b R = Me R' = H
1c R = Me R' = Ac
1d R = H R' = OH

Finally, fraction C, composed of free fatty acids, was esterified with ethereal diazomethane and rechromatographed on a Si gel column to afford a mixture of methyl hexadec-11*Z*-enoate (**2**) [1] and methyl hexadeca-7*Z*,10*Z*,13*Z*-trienoate (**3**) [2], subsequently separated by preparative silver nitrate-TLC, and methyl (10*R*)-hydroxyhexadeca-7*Z*,11*E*,13*Z*-trienoate (**1b**). The first two esters, already known, were identified on the basis of their IR, mass spectral and ^{1}H NMR features. ^{13}C NMR data (Table 1) confirmed the *Z* configuration of double bonds according to Bus *et al.* [3] and Gunstone *et al.* [4].

Hydroxy methyl ester **1b**, $[\alpha]_D + 13°$, showed in its IR spectrum peaks attributable to a hydroxyl group (3610, 3460 cm^{-1}), an ester carbonyl group (1730 cm^{-1}) and conjugated (1685 cm^{-1}) and isolated (1640 cm^{-1}) double bonds. Mass spectral analysis gave fragments *m/z* 280 $[M]^+$ (4%), 262 $[M-H_2O]^+$ (25), 207 $[M-CH_2CO_2Me]^+$ (100), 203 $[M-H_2O-CO_2Me]^+$ (35), 189 $[M-H_2O-CH_2CO_2Me]^+$ (18), 161 $[M-H_2O-(CH_2)_3CO_2Me]^+$ (21), 147 $[M-H_2O-(CH_2)_4-CO_2Me]^+$ (31), 133 $[M-H_2O-(CH_2)_5CO_2Me]^+$ (31). The chemical shifts and couplings in the ^{1}H NMR spectra of **1b** (Table 2) suggested the presence of an *E*,*Z* conjugated diene which was confirmed by spin decoupling experiments. Irradiation at δ 6.51 collapsed the signals at 5.97 and 5.69 to two doublets, while irradiation at 5.97 altered the signals at 6.51 and 5.56 into a doublet and a triplet, respectively. The position of the diene system, as well as that of the hydroxyl group, was assigned by irradiation at δ 2.07 and 5.69. In the first case the terminal methyl and the olefinic proton at δ 5.56 were simplified into a singlet and a doublet, respectively, showing the diene system to be at the 11,13 positions. Similarly the simplification of the hydroxyl *gem* proton signal allowed the assignment of the hydroxyl group at C-10. ^{13}C NMR of the corresponding acetate **1c** (Table 1) and selective decoupling experiments confirmed such a partial structure and fixed the third double bond at C-7 with the *Z* configuration. All the chemical shifts were confirmed by comparison with those of methyl ricinoleate [5] and using corrective parameters, such as the influence of carbomethoxy group [3], of the double bonds [4] and the effect of an acetyl group [6]. Finally, the absolute configuration *R* at C-10 was assigned on the basis of the specific rotations of the saturated derivatives of **1b** and **1c**. Catalytic hydrogenation of these compounds gave methyl esters **4a** and **4b**, $[\alpha]_D + 7°$ and $[\alpha]_D - 5°$, respectively.

These specific rotations compared with those available for the homologous series of saturated hydroxy fatty acid methyl esters [7] showed an *S* configuration at C-10 for **4a** and **4b** and, therefore, an *R* configuration for **1b** and **1c**.

From a biogenetic point of view, **1a** could arise from the



1b R = H **4a** R = H

1c R = Ac **4b** R = Ac

Table 1. ^{13}C NMR data of methyl esters **1c**, **2** and **3** in $CDCl_3$ (TMS as internal standard)

	1c	2	3		1c	2	3
C-1	174.37 *s*	173.87 *s*	174.02 *s*	C-10	74.30 *d*	27.20 *t*	128.22 *d*
C-2	34.13 *t*	34.18 *t*	34.09 *t*	C-11	130.54 *d*	129.86 *d*	128.36 *d*
C-3	24.98 *t*	24.86 *t*	24.92 *t*	C-12	133.91 *d*	129.92 *d*	25.74 *t*
C-4	29.54 *t*	29.17 *t**	28.81 *t*	C-13	128.21 *d*	27.10 *t*	127.36 *d*
C-5	29.74 *t*	29.32 *t**	29.35 *t*	C-14	127.64 *d*	32.07 *t*	131.88 *d*
C-6	27.81 *t*	29.41 *t**	27.11 *t*	C-15	20.75 *t*	22.49 *t*	20.65 *t*
C-7	134.77 *d*	29.41 *t**	129.99 *d*	C-16	14.12 *q*	14.08 *q*	14.30 *q*
C-8	123.15 *d*	29.49 *t**	128.07 *d*	OMe	51.45 *q*	51.38 *q*	51.47 *q*
C-9	32.50 *t*	29.81 *t**	25.74 *t*	OCOMe	170.31 *s*, 21.31 *q*	—	—

*Interchangeable values

Table 2. ^{1}H NMR data of methyl ester **1b** in $CDCl_3$ (TMS as internal standard)

H-2	2.30 *t*	H-11	5.69 *dd*
H-3	1.62 *m*	H-12	6.51 *dd*
H-4, H-5	1.31 *m*	H-13	5.97 *dd*
H-6	2.17 *m*	H-14	5.56 *m*
H-7, H-8	5.42 *m*	H-15	2.07 *m*
H-9	2.30 *m*	H-16	0.97 *t*
H-10	4.21 *m*	OMe	3.66 *s*

J (Hz): 2, 3 = 7.8; 10, 11 = 7.6; 11, 12 = 15.1; 12, 13 = 11.0; 13, 14 = 10.8; 14, 15 = 8.1; 15, 16 = 7.6.

corresponding hydroperoxide, **1d**. Hydroperoxide formation, via enzymatic oxidation or autoxidation for differently unsaturated C_{18}-fatty acids, has been extensively studied *in vitro*. The enzymatic oxidation of methyl linolenate [8], homologous to **1b**, gave two isomeric allylic hydroperoxide trienes while autoxidation yielded not only a mixture of positional isomers but also racemic mixtures [9]. The isolation of an enantiomeric pure alcohol from *L. minor* seems, therefore, to exclude **1a** as an artefact arising from autoxidation of hexadeca-7*Z*,10*Z*,13*Z*-trienoic acid during the chomatography [10] and suggests the presence of an highly regioselective enzymatic system.

EXPERIMENTAL

^{1}H (270 MHz) and ^{13}C NMR (67.88 MHz) spectra were obtained from $CDCl_3$ solns using TMS as int. standard at the Centro di Metodologie Chimico Fisiche of the University (I. Giudicianni).

Isolation of lipid fractions. Air dried plants of *L. minor* (85 g) were extracted continuously with cold Et_2O to give, after removal of solvent, a crude product (2.5 g) which was directly chromatographed on a Si gel column. Elution with petrol (1.5.1.) gave a mixture of non-polar hydrocarbons (300 mg), NMR: δ 0.92, 1.22, 1.63 and 5.37; IR ν_{max} significant absorption at 3050–2800 and 1670–1630 cm^{-1}. Petrol–Et_2O (9:1, 2 l.) gave a mixture of triacylglycerols (1.2 g); finally elution with petrol–Et_2O (1:1, 1.2 l.) gave a free fatty acid fraction (200 mg).

Triacylglycerols. The crude mixture of triacylglycerols, NMR: δ 4.15 (*dd*), 4.30 (*dd*), 5.28 (*m*), was refluxed with C_6H_6 (5 ml) and 0.5 M NaOMe in MeOH (14 ml). After 1 hr Et_2O was added and the organic layer washed with H_2O until neutral. Evaporation of the solvent furnished a mixture (1 g) of methyl esters. HPLC analysis of the mixture (Micropack-5, eluent: hexane–EtOH, 99:1, flow rate 3 ml/min) showed the presence of methyl esters **1b**, **2** and **3** in a ratio of 1.2:3.0:4.1.

Free fatty acids. The fraction containing free fatty acids was treated with CH_2N_2–Et_2O and the crude esterification product chromatographed on Si gel. Elution with C_6H_6 gave a mixture of **2** and **3** (160 mg), which was separated by prep. $AgNO_3$ TLC (Si gel impregnated with 20% $AgNO_3$; eluent: hexane–Et_2O, 3:2). Elution with C_6H_6–Et_2O (4:1) gave hydroxy ester **1b** $[\alpha]_D + 13°$ (*c* 1.0) (22 mg). Acetylation with Ac_2O in dry pyridine afforded acetate **1c** $[\alpha]_D - 10°$ (*c*15), ^{1}H NMR: δ 2.04 (3H, *s*, MeCOO–), 5.47 (1H, *m*, H-10).

Hydrogenation of **1b** *and* **1c**. Pure samples (20 mg) of each in 95% EtOH (2 ml) were hydrogenated with PtO_2 at atm. pres. for 30 min. Usual work-up of the reaction mixture gave pure satd derivatives **4a** $[\alpha]_D + 7°$ (*c* 0.8) and **4b** $[\alpha]_D - 5°$ (*c* 1.0).

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