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OXYGENATED FATTY ACIDS FROM *LEMNA TRISULCA**

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Key Word Index *Lemna trisulca*; Lemnaceae; oxygenated fatty acids; structural determination.

Abstract— (12*S*)-Hydroxyhexadeca-8*Z*,10*E*,14*Z*-trienoic acid and a prostaglandin-like C_{16} fatty acid have been isolated from the acidic fraction of *Lemna trisulca* together with several other unsaturated fatty acids.

INTRODUCTION

In a continuation of our study of the chemical composition of aquatic plants growing in Southern Italy [1] we have now examined *Lemna trisulca*, a perennial species widely distributed in swamps with a remarkable adaptation to the aquatic environment. Specimens of *L. trisulca* were collected in the summer in irrigation canals around Naples where other aquatic plants are also present.

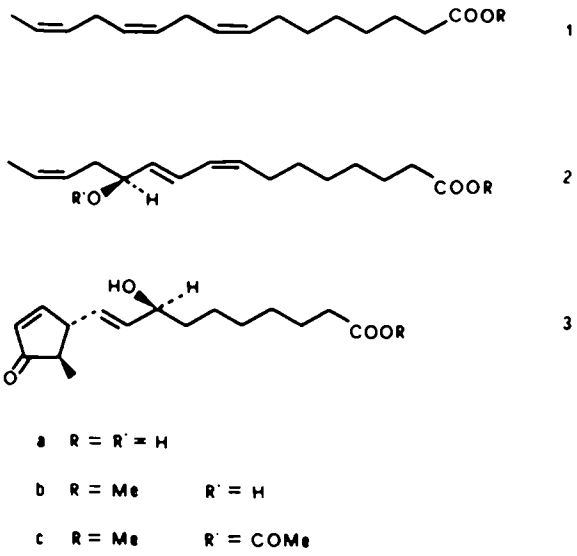
RESULTS AND DISCUSSION

Homogenized and lyophilized plants were extracted with aqueous acetone at room temperature. The extract was evaporated *in vacuo* and divided between water and ethyl acetate. The organic layer was treated with aqueous NaOH and the aqueous phase, after reacidification with 2 N H_2SO_4, was extracted with ethyl acetate to give a mixture of acidic compounds.

Column chromatography on HCl-washed silica gel and elution with mixtures of benzene and ethyl acetate gave three main fractions. Fraction A (benzene–ethyl acetate, 9:1) was esterified with ethereal CH_2N_2 to give a mixture of variously unsaturated fatty acid methyl esters which were separated by prep. silver nitrate TLC: the major components were identified as methyl hexadec-11*Z*-enoate [2], methyl hexadeca-8*Z*,11*Z*-dienoate [3] and methyl hexadeca-8*Z*,11*Z*,14*Z*-trienoate (**1b**) mainly on the basis of their ^{13}C NMR and mass spectra.

Fraction B (benzene–ethyl acetate, 4:1) consisted of an unsaturated hydroxy acid which was purified as the methyl ester and identified as methyl (12*S*)-hydroxyhexadeca-8*Z*,10*E*,14*Z*-trienoate (**2b**), $[\alpha]_D + 5°$. Its IR spectrum showed peaks attributable to an hydroxyl group (3610, 3450 cm^{-1}), an ester carbonyl group (1735 cm^{-1}), and conjugated (1640 cm^{-1}) and isolated (1685 cm^{-1}) double bonds. The UV spectrum in EtOH showed a strong absorption at 233 nm. Mass spectral analysis gave fragments at *m/z* 280 $[M]^+$ (4%), 262 $[M - H_2O]^+$ (28), 207 $[M - CH_2CO_2Me]^+$ (100), 203 $[M - H_2O - CO_2Me]^+$ (38), 189 $[M - H_2O - CH_2CO_2Me]^+$ (57), 133 $[M - H_2O - (CH_2)_5CO_2Me]^+$ (70). The two-dimensional proton homonuclear chemical shift correlation spectrum allowed us to obtain unambiguous data on the structure of **2b**. The H-16 methyl was coupled with the H-15 and H-14 vinylic protons which were correlated with the H-13 methylene. The OH geminal H-12 methine was coupled with the H-13 methylene and the H-11 olefinic protons and was correlated with the H-10 one. Clear correlations were then present among the H-8, H-9, H-10 and H-11 olefinic protons. Upon irradiating the multiplet at δ5.42 the H-16 methyl appeared as a singlet; irradiation at δ5.54 collapsed the H-9 double doublet into a doublet (J = 11 Hz) whereas irradiation at δ6.49 modified the H-9 and H-11 double doublets into two doublets (J = 10.6 and 7.8 Hz, respectively). Finally irradiation of the H-12 methine at δ4.18 still transformed the H-11 double doublet into a doublet (J = 15.0 Hz). The chemical shifts and the J values suggested a conjugated diene with a 8*Z*,10*E* configuration. The ^{13}C NMR spectrum of the corresponding acetate (**2c**) as well as decoupling experiments confirmed such a partial structure and fixed the third double bond with a *Z* configuration at C-14.

The absolute configuration at C-12 was assigned on the basis of the comparison of the specific rotation of the



* Part 5 in the series "Studies on Aquatic Plants Distributed in Italy". For part 4 see ref. [1].

Table 1. ^{13}C NMR data of fatty acid methyl esters **1b**, **2c** and **3b**

	1b	2c	3b
C-1	174.43 *s*	174.30 *s*	174.38 *s*
C-2	34.16 *t*	34.20 *t*	34.06 *t*
C-3	24.97 *t*	25.05 *t*	24.91 *t*
C-4	29.20 *t*•	29.25 *t*•	29.39 *t*
C-5	29.03 *t*•	29.40 *t*•	29.69 *t*
C-6	29.71 *t*	29.80 *t*	25.62 *t*
C-7	27.28 *t*	27.75 *t*	37.19 *t*
C-8	130.12 *d*	127.01 *d*	72.65 *d*
C-9	128.06 *d*†	128.39 *d*†	135.22 *d*
C-10	25.74 *t*	134.87 *d*	130.18 *d*
C-11	128.35 *d*†	130.74 *d*	52.02 *d*
C-12	128.30 *d*†	74.42 *d*	163.43 *d*
C-13	25.44 *t*	32.55 *t*	132.98 *d*
C-14	128.89 *d*	123.75 *d*	210.91 *s*
C-15	124.15 *d*	128.01 *d*†	45.11 *d*
C-16	12.78 *q*	12.84 *q*	13.48 *q*
OMe	51.34 *q*	51.35 *q*	51.30 *q*
OCOMe		170.35 *s*	
		21.29 *q*	

*,†Assignments bearing the same superscript may be reversed.

Table 2. ^1H NMR data of fatty acid methyl esters **2b** and **3b**

	2b	3b		2b	3b
H-2	2.30 *m*	2.27 *t*	H-10	6.49 *dd*	5.54 *m*
H-3	1.62 *m*	1.61 *m*	H-11	5.65 *dd*	3.21 *m*
H-4	1.31 *br*	1.31 *br*	H-12	4.18 *m*	7.39 *dd*
H-5	1.31 *br*	1.31 *br*	H-13	2.30 *m*	6.08 *dd*
H-6	1.31 *br*	1.31 *br*	H-14	5.42 *m*	
H-7	2.07 *m*	1.45 *m*	H-15	5.42 *m*	2.13 *m*
H-7•	2.07 *m*	1.53 *m*	H-16	1.68 *d*	1.03 *d*
H-8	5.54 *m*	4.00 *m*	OMe	3.66 *s*	3.63 *s*
H-9	6.01 *dd*	5.54 *m*			

saturated derivative methyl ester, $[\alpha]_D + 6°$ with those of the homologous series of hydroxy fatty acid methyl esters [4].

Fraction C (benzene ethyl acetate, 3:2) consisted mainly of a crude product which was purified by prep. TLC. Esterification with CH_2N_2 gave an oil, $[\alpha]_D$ $+21°$, which was assigned structure **3b**. The UV spectrum showed a strong absorption at 221 nm, indicative of a carbonyl group conjugated with a double bond. Absorptions in the IR spectrum at 3430, 1735, 1705, 1670 and 1595 cm^{-1} indicated the presence of a hydroxyl group, an ester carbonyl group, a conjugated five-membered ring carbonyl and an isolated *trans* double bond. The mass spectrum gave significant fragmentions at *m/z* 294 $[M]^+$ (35%), 279 $[M - CH_3]^+$ (26), 276 $[M - H_2O]^+$ (32), 221 $[M - CH_2CO_2Me]^+$ (15), 203 $[M - H_2O - CH_2CO_2Me]^+$ (25), 179 $[M - (CH_2)_4CO_2Me]^+$ (16), 161 $[M - H_2O - (CH_2)_4CO_2Me]^+$ (100), 95 $[C_6H_7O]^+$ (27) and 81 $[C_5H_5O]^+$ (31). The ^1H NMR spectrum showed olefinic signals centred at δ5.54, 6.08 and 7.39, an OH geminal methine at δ4.00, a secondary methyl at δ1.03 besides the OMe singlet at δ3.63. Irradiation of the double doublet at δ6.08 collapsed the signal at δ7.39 (*dd*) into a doublet (J = 2.4 Hz) and modified the methine multiplet centred at δ3.21. Upon irradiating at δ7.39 the signal at δ6.08 become a doublet (J = 2 Hz) and the signal at δ3.21 was still modified. Irradiation of the H-11 proton at δ3.21 transformed the vinylic protons at δ6.08 and 7.39 into two doublets (J = 6.1 Hz) and modified the H-10 signal at δ5.54. Finally irradiation at δ5.54 simplified the methine signals at δ3.21 and δ4.00. The ^1H NMR spectrum of **3b** in the presence of $Eu(DPM)_3$ showed the H-9 and H-10 signals shifted to δ8.36 and 8.05, respectively, as double doublets. Irradiation of the H-8 proton collapsed the H-9 signal into a doublet (J = 15.1 Hz) whereas irradiation of the H-11 proton modified the H-10 one into a doublet thus showing an *E* configuration at C-9. NOE difference experiments carried out in the standard manner [5] saturating the H-16 methyl protons showed effects with the H-11 and H-15 methines thus suggesting a *trans* configuration between these protons. The ORD spectrum of **3b** showed a strongly positive Cotton effect indicating an α configuration at C-11, the same as that in the corresponding C-12 position of prostaglandin A [6].

As far as the C-8 configuration is concerned, De Clerq *et al.* [7] described a difference between the H-13 and H-14 chemical shifts in the (15*R*) and (15*S*) series of prostaglandins: the close values of the H-9 and H-10 protons with the corresponding H-14 and H-13 protons of PGA_2 [8] suggested an (8*S*) configuration for **3b**. A comparison of the ^{13}C chemical shifts of **3b** with those of prostaglandins [9] confirmed the assigned structure.

The possibility that these oxygenated fatty acids were artefacts produced during extraction and separation procedures was ruled out by minimizing exposure to light and air during the extraction and by monitoring their presence in the crude acetone extract by HPLC.

From a biogenetic point of view **2a** and **3a** are presumably formed from hexadecatrienoic acid (**1a**) through two different enzymic systems: a lipoxygenase reductase pathway giving rise to the hydroxytrienoic acid (**2a**) whereas the action of a cyclooxygenase forms the hydroxydienonic acid (**3a**). The similarity of **2a** and **3a** to HETEs and PGs, respectively, as well as the similarity of **3a** to a hydroxycyclopentenone fatty acid isolated from another aquatic plant *Eleocharis microcarpa* which inhibited the growth of blue-green algae [10] suggested further tests on the biochemical effects of **2a** and **3a**. Preliminary bioassays of crude samples of fraction B and C against the algae *Anabaena cylindrica* and *Anacystis nidulans* showed a good growth inhibition for **3a** whereas **2a** was inactive. Further work is, however, necessary to prove the allelochemical properties of these compounds.

EXPERIMENTAL

^1H and ^{13}C NMR were measured at 270 and 67.88 MHz, respectively, in $CDCl_3$. The deuterium resonance of the $CDCl_3$ was used as lock signal. Two-dimensional proton homonuclear chemical shift correlation expts were performed with a microprogram using a 256 × 1024 data point matrix, transforming 1024 data points in both domains, spectral width 1200 Hz in both domains. MS were recorded at 12 eV with the source of 150°. *L. trisulca* was identified by Prof. G. Aliotta, University of Naples.

Isolation of acidic components. Lyophilized plants (900 g) of *L. trisulca* were exaustively extracted with aq. Me_2CO and the Me_2CO soln concd *in vacuo*. Partition between H_2O and EtOAc gave an organic layer which was treated × 2 with aq. NaOH (10%). The aq. phase, after reacidification with 2 N H_2SO_4, was again extracted with EtOAc to give a crude mixture of acidic components (550 mg). CC on HCl washed silica gel (55 g). gave three main fractions.

Fraction A. Eluted with C_6H_6 EtOAc (9:1); 550 ml; 150 mg was esterified with CH_2N_2-Et_2O and sepd by prep. $AgNO_3$ TLC (20% $AgNO_3$), hexane–Et_2O (3:2), into three C_{16} Me esters identified as the already known Me hexadeca-11*Z*-enoate and Me hexadeca-8*Z*,11*Z*-dienoate on the basis of their MS, ^{13}C and ^1H NMR data and Me hexadeca-8*Z*,11*Z*,14*Z*-trienoate (**1b**): MS: *m/z* 264 $[M]^+$ (8%), 205 $[M-CO_2Me]^+$ (30), 191 $[M-CH_2CO_2Me]^+$ (100), 135 $[M-(CH_2)_5-CO_2Me]^+$ (64); ^1H NMR: δ 5.41 (*m*, 6H), 3.63 (*s*, 3H), 2.76 (*t*, 4H) 2.30 (*t*, 2H), 2.06 (*m*, 2H), 1.65 (*d*, 3H).

Fraction B. Eluted with C_6H_6–EtOAc (4:1); 220 ml; 40 mg was esterified with CH_2N_2 to give crude **2b** which was purified by prep. TLC in petrol–Et_2O (9:1). Compound **2b** (25 mg) had $[\alpha]_D$ + 5° (*c* = 0.9, $CHCl_3$), UV λ_{max} 233 nm (ε 24000 in EtOH). Pure **2b** (10 mg) was treated overnight with Ac_2O (0.01 ml) in dry pyridine (1 ml) at room temp. Usual work-up gave the acetyl derivative **2c** $[\alpha]_D$ − 12° (*c* = 0.7); ^1H NMR δ 2.04 (*s*, 3H), 5.45 (*m*, 1H). A pure sample of **2b** (10 mg) in 95% EtOH (1.3 ml) was hydrogenated with PtO_2 at atm. press. for 30 min to give the corresponding hexahydroderivative $[\alpha]_D$ + 6° (*c* = 0.8).

Fraction C. Eluted with C_6H_6–EtOAc (3:2); 330 ml; 25 mg consisted of a single compound which was purified by prep. TLC on silica gel in hexane–Et_2O HCO_2H (25:25:1). Esterification with CH_2N_2 Et_2O gave **3b**: $[\alpha]_D$ + 21° (*c* = 0.8 in $CHCl_3$); UV λ_{max} 221 (ε 10700 in EtOH).

HPLC analysis of crude extract. A dried sample of the Me_2CO extract was esterified with CH_2N_2 and the presence of **2b** and **3b** was checked by HPLC using a Micropack Si-5 column and hexane–EtOH (99:1) as eluant. Compounds were detected using UV absorption.

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