crystalline form); PC: R_fs 0.75 (solvent 1); 0.43 (solvent 2); RR_ss on the amino acid analyser (system I); 0.86 and 1.19 (relative to serine and aspartic acid, respectively); CI/MS (isobutane, probe) 200 eV, *m/z* (rel. int.): 164 $[M+1]^+$ (16), 116 $[M-47]^+$ (100); EI/MS (GC) of TMSi-1: 20 eV, *m/z* (rel. int.): 379 $[M]^+$ (0.6), 364 $[M-15]^+$ (9), 320 $[TMSiOCHCH(NHTMSi)COOTMSi]^+$ (3), 291 $[M-OTMSi]^+$ (68), 262 $[M-COOTMSi]^+$ (29), 219 $[TMSiNH{=}CHCOOTMSi+1]^+$ (100), 218 $[TMSiNH{=}CHCOOTMSi]^+$ (93), 161 $[CH_3CH_2OCH_2CHOTMSi]^+$ (21); ^{13}C NMR (25.05 MHz, D_2O, TMS): δ14.3 (*q*, C-2'), 58.0 (*d*, C-2), 67.4 (*t*, C-1'), 67.9 (*d*, C-3), 71.1 (*t*, C-4), 171.4 (*s*, C-1); ^1H NMR (100 MHz, D_2O, TMS): δ1.20 (3H, *t*, J = 7 Hz, H-2'), 3.56 (2H, *q*, J = 7 Hz, H-1'), 3.67 (2H, partial overlap with H-1', H-4), 3.93 (1H, *d*, J = 4 Hz, H-2), 4.28 (1H, *dd*, J = 4, 4 Hz, H-3); IR ν_{max}^{KBr} cm^{-1}: 1050 (*m*), 1120 (*s*), 1380 (*s*), 1570 (*s*), 1630 (*s*), 1660 (*s*), 3400 (*s*).

Chemical degradation. Reduction with HI–red P was carried out according to ref. [3]. The reduction products were separated by prep. PC with solvent 2 and determined by GC/MS and the amino acid analyses (system I). The yields of the products were as follows: **2**:**3**:**4** = 0.5:0.3:0.1 [molar ratios to original **1** (1.0)].

Determination of the stereochemical properties of the products **2** *and* **3**. The stereoisomers of the product **2** were determined by the amino acid analyser (system I); RR_ss (relative to L_s-serine): the product **2**; 0.71, synthetic D_s- and L_s-*erythro*-**2**; 0.71, synthetic D_s- and L_s-*threo*-**2**; 0.69. The optical properties of the products **3** was examined by the chromatographic method of ref. [4] using the analytical system described previously [5]; RR_ss (relative to L_s-leucine): L_s-leucyl peptide of the product **3**, 1.13; synthetic L_s-leucyl-D_s-**3**, 1.13; synthetic L_s-leucyl-L_s-**3**, 1.61.

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ACETOGENINS FROM THE AQUATIC PLANT *ELODEA CANADENSIS*

LUCIO PREVITERA, DOMENICO MEROLA and PIETRO MONACO

Department of Organic and Biological Chemistry of the University, Via Mezzocannone 16, 80134 Napoli, Italy

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Key Word Index—*Elodea canadensis*; Hydrocharitaceae; acetogenins.

Abstract—13-(2-furyl)-Tridec-12*E*-en-1-yne and (7*S*)-hydroxyhexadeca-8*E*,10*Z*,13*Z*-trienoic acid have been isolated from *Elodea canadensis* in addition to the already known 13-(2-furyl)-tridec-1-yne, hexadec-11*Z*-enoic, hexadeca-7*Z*,10*Z*,13*Z*-trienoic and (10*R*)-hydroxyhexadeca-7*Z*,11*E*,13*Z*-trienoic acids.

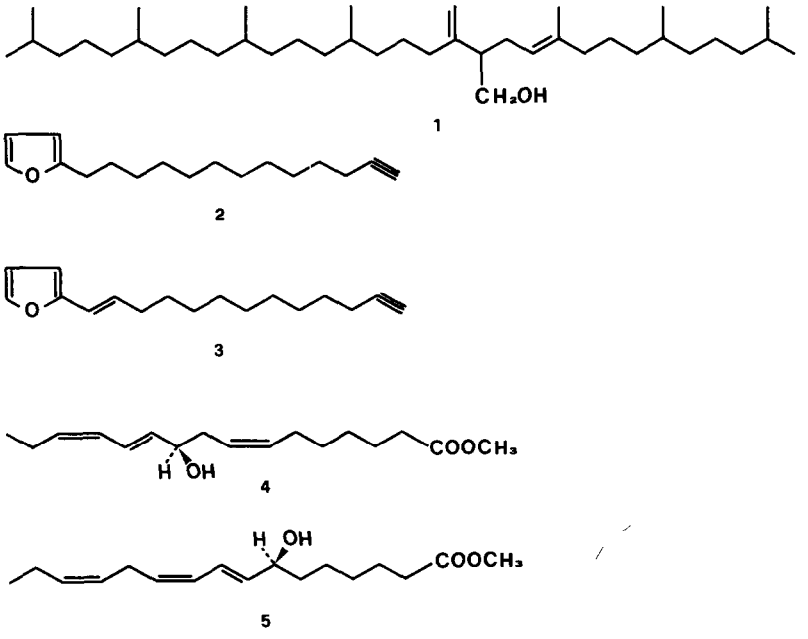
INTRODUCTION

In connection with a systematic study [1] of the aquatic species distributed in Italy, we recently described the isolation of a novel tetraterpene alcohol (**1**) [2] from *Elodea canadensis*. In this paper we now report the chemical investigation of other metabolites from the same source.

RESULTS AND DISCUSSION

The plants, collected in the Botanical Garden of the University of Naples, were air-dried and extracted with cold ether to afford a residue that, after treatment with charcoal, was separated into acidic and neutral fractions by conventional procedures.

The neutral fraction was chromatographed on neutral alumina to give three groups of fractions. The first group, eluted with petrol, consisted of two compounds (**2** and **3**) which were separated by prep. TLC. Compound **2** showed spectroscopic feature identical with those reported for the already known avocadynofuran [3]. Compound **3** had a molecular peak at *m/z* 244 and absorptions in the IR spectrum at 3280, 2100, 1590, 1500, 1260, 1145 and 1050 cm^{-1}. The ^1H NMR spectrum showed three furanic



protons at δ6.17 (1H, *d*, J = 3.3 Hz), 6.33 (1H, *dd*, J = 1.8 and 3.3 Hz) and 7.30 (1H, *d*, J = 1.8 Hz), two protons of an *E*-double bond as a doublet and a multiplet centred at 6.14 (J = 16.2 Hz), four protons as a multiplet centred at 2.17 attributable to two methylenes linked to an olefinic and an acetylenic carbon, an acetylenic proton at 1.92 as a triplet and, finally, seven methylenes as a broad peak at 1.26. The second group of fractions, eluted with petrol–diethyl ether (19:1), gave tetraterpene (**1**) and phytol, whereas the third group, eluted with a mixture (4:1) of the same solvents, consisted of sitosterol and triacylglycerols ^{1}H NMR: δ4.15 (*dd*), 4.30 (*dd*) and 5.28 (*m*)].

The acidic fraction was esterified with ethereal diazomethane and chromatographed on silica gel. Petrol eluted a mixture of methyl hexadec-11*Z*-enoate [4] and methyl hexadeca-7*Z*,10*Z*,13*Z*-trienoate [5] which was resolved by silver nitrate–silica gel prep. TLC. Pure methyl esters were compared (^{1}H NMR, ^{13}C NMR and mass spectra) with authentic samples. Petrol–diethyl ether (9:1) gave a complex mixture of isoprenoidic compounds which have not yet investigated and, finally, petrol–diethyl ether (4:1) eluted two isomeric hydroxymethyl esters (**4** and **5**) which were separated by prep. TLC.

Compound **4**, $[\alpha]_D$+13°, was identified by comparison with authentic material as methyl (10*R*)-hydroxyhexadeca-7*Z*,11*E*,13*Z*-trienoate which we recently isolated from the aquatic plant *Lemna minor* [6]. Hydroxymethyl ester (**5**), $[\alpha]_D$ +2°, showed in its mass spectrum fragments at *m/z* 280 $[M]^+$ (7), 262 $[M-H_2O]^+$ (30), 225 $[M-C_4H_7]^+$ (35), 207 $[M-CH_2COOMe]^+$ (100), 203 $[M-H_2O-COOMe]^+$ (40), 189 $[M-H_2O-CH_2COOMe]^+$ (16) and 161 $[M-H_2O-(CH_2)_3COOMe]^+$ (25). The IR spectrum had absorptions at 3610, 3470, 1730, 1690 and 1640 cm^{-1}. The ^{1}H NMR spectrum showed signals at δ0.98 (3H, *t*), 1.31 (4H, *s*), 1 61 (2H, *m*), 2.08 (2H, *m*), 2.30 (2H, *t*, J = 7.3 Hz), 2.93 (2H, *dd*, J = 6.9 and 7.3 Hz), 3.66 (3H, *s*), 4.16 (1H, *m*), 5.40 (3H, *m*), 5.68 (1H, *dd*, J = 15.1 and 13.2 Hz), 5.98 (1H, *dd*, J = 11.0 and 10.6 Hz) and 6.51 (1H, *dd*, J = 15.1 and 11.0 Hz). Irradiation at δ6.51 collapsed signals at δ5.68 and 5.98 to two doublets while irradiation at δ5.98 collapsed signals at δ6.51 to a doublet and altered the multiplet at δ5.40 significantly. Irradiation at δ5.68 collapsed signals at δ4.16 and 6.51 to a triplet and a doublet, respectively. Finally, irradiation at δ5.40 simplified a signal at δ5.98 to a doublet, the methylene signal at δ2.93 to a singlet and the methylene at δ2.08 to a quadruplet. The ^{13}C NMR spectrum (Table 1) confirmed the assigned structure of methyl (7*S*)-hydroxyhexadeca-8*E*,10*Z*,13*Z*-trienoate. Acetylation of **5** with acetic anhydride in dry pyridine gave the corresponding acetate $[\alpha]_D$ −9°. The absolute configuration at C-7 of **5** was assigned as *S* on the basis of the specific rotation, $[\alpha]_D$ −4°, of the corresponding saturated derivative. This value, compared with those of the homologous series of hydroxy fatty acid methyl esters [7], showed the *R*-configuration for the saturated hydroxy ester and, therefore, the *S*-configuration for **5**.

Table 1 ^{13}C NMR spectral data of the methyl ester, **5**

C-1	174.37 (*s*)	C-10	126.47 (*d*)
C-2	34.04 *t*)	C-11	125.46 (*d*)
C-3	24.87 (*t*)	C-12	25.95 (*t*)
C-4	29.65 (*t*)	C-13	127.75 (*d*)
C-5	25.30 (*t*)	C-14	132.38 (*d*)
C-6	37.26 (*t*)	C-15	20.92 (*t*)
C-7	72.79 (*d*)	C-16	14.17 (*q*)
C-8	130.69 (*d*)	OMe	51.41 (*q*)
C-9	136.28 (*d*)		

EXPERIMENTAL

^{1}H NMR (270 MHz) and ^{13}C NMR (67.88 MHz) spectra were obtained from $CDCl_3$ using TMS as int. standard at the Centro di Metodologie chimicofisiche of this university (I. Giudicianni). The plants of *E. canadensis* were identified by G. Aliotta, Professor of Botany at the University of Naples. The Et_2O extract of air-dried plants (120 g) was treated with charcoal and then evaporated under red. pres. to afford a crude product (2.1 g) which was separated by conventional procedures into acidic and neutral fractions.

Isolation of the neutral components. The neutral fraction (1.3 g) was chromatographed on neutral alumina. Elution with petrol (120 ml) gave a mixture of **2** and **3** which was resolved by prep. TLC (petrol). Compound **2** (10 mg) has MS *m/z*: 246, 133, 119, 107, 95, 81; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3285, 2105, 1595, 1505, 1260, 1140, 1050; ^{1}H NMR: δ1.25, 1.92, 2.12, 2.58, 5.94, 6.24, 7.25. Compound **3** (15 mg) had MS *m/z*: 244, 107, 94, 81.

Petrol–Et_2O (19:1; 240 ml) gave tetraterpene (**1**) (35 mg) and phytol (145 mg). Petrol–Et_2O (4:1; 600 ml) gave sitosterol (280 mg) and a mixture of triacylglycerols (410 mg) which was refluxed with C_6H_6 (10 ml) and 0.5 M NaOMe in MeOH (20 ml) to give a mixture of methyl esters (354 mg). HPLC analysis of such a mixture (Micropack-5; hexane–EtOH, 99: 1; flow rate 4 ml/min) showed the presence of **4**, **5** and the methyl esters of 16.1 and 16:3 fatty acids in a 1.0:2.1:3.4:5.2 ratio.

Isolation of the acidic components. The acidic fraction (600 mg) was treated with ethereal CH_2N_2 (excess) and then absorbed on silica gel. Elution with petrol (60 ml) afforded a mixture (120 mg) of 16:1 and 16:3 fatty acids methyl esters. Petrol–Et_2O (9:1; 60 ml) gave a mixture of isoprenoidic compounds (105 mg) and, finally, petrol–Et_2O (4:1; 30 ml) gave a mixture of **4** and **5** which was resolved by prep. TLC (petrol–Et_2O, 9:1; two runs). Compound **4** (21 mg) had: $[\alpha]_D$ +13° (*c* 0.8); MS *m/z*: 280, 262, 207, 203, 189, 161, 147, 133; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3610, 3460, 1730, 1685, 1640. ^{1}H NMR: δ0.97, 1.31, 1.62, 2.07, 2.17, 2.30, 3.66, 4.21, 5.42, 5.56, 5.69, 5.97, 6.51. Compound **5** (28 mg) had $[\alpha]_D$ +2° (*c* 1.0).

Acetylation of **5**. Pure compound **5** (10 mg) was treated with Ac_2O (0.01 ml) in dry C_5H_5N (1 ml) overnight. Usual work-up gave the acetyl derivative, $[\alpha]_D$ −9° (*c* 0.7). ^{1}H NMR: δ2.04 (3H, *s*), 5.47 (1H, *m*).

Hydrogenation of **5**. A pure sample of **5** (12 mg) in 95% EtOH (1.5 ml) was hydrogenated with PtO_2 at atm. pres. for 30 min to give the corresponding hexahydro derivative, $[\alpha]_D$ −4° (*c* 1.1).

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