Journal of Natural Products
Vol. 50, No. 5, pp. 807-810, Sep-Oct 1987

FURTHER OXYGENATED FATTY ACIDS FROM *LEMNA MINOR*[1]

LUCIO PREVITERA and PIETRO MONACO

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

ABSTRACT.—In a re-investigation of the aquatic plant *Lemna minor*, two novel cyclopentanoid C_{16} fatty acids, **6a** and **7a**, have been isolated and characterized as the corresponding methyl esters on the basis of their physical and chemical features. A biogenetic pathway similar to that of the prostaglandins is suggested for these compounds.

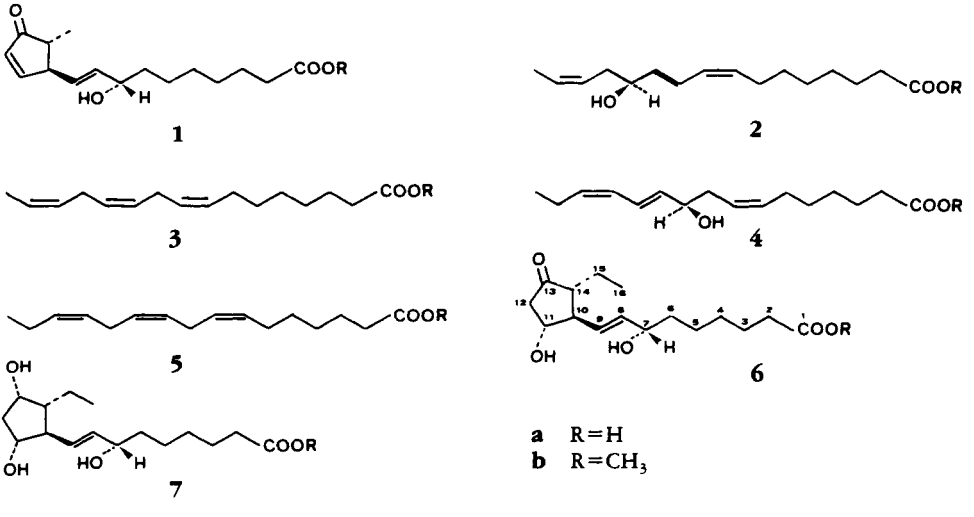
In the course of our investigation of the chemical composition of aquatic plants growing in Italy, we have recently isolated from *Lemna trisulca* L. (1) the C_{16} hydroxycyclopentenonic acid **1a**, 12*S*-hydroxyhexadeca-8*Z*,10*E*,14*Z*-trienoic acid [**2a**], and hexadeca-8*Z*,11*Z*,14*Z*-trienoic acid [**3a**]. The structural features of **1a** and **2a** suggested the hypothesis that they might arise from **3a** in a similar way to the formation of prostaglandins (PGs) and hydroxyeicosatetraenoic acids (HETEs) from arachidonic acid in mammals (2,3).

In a previous report (4) on *Lemna minor* L. (Lemnaceae), we isolated 10*R*-hydroxyhexadeca-7*Z*,11*E*,13*Z*-trienoic acid [**4a**] and hexadeca-7*Z*,10*Z*,13*Z*-trienoic acid [**5a**]. The possibility of also finding cyclic fatty acids in this species, suggesting the existence of enzymic systems able to produce these compounds, prompted us to reinvestigate *L. minor*, and we now describe the characterization of two prostaglandin-like fatty acids **6a** and **7a**.

DISCUSSION

An Me_2CO extract of homogenized and lyophilized plants was distributed between H_2O and EtOAc and the organic layer treated with 2N aqueous NaOH. Re-acidification of the aqueous phase with 2N H_2SO_4 and extraction with EtOAc gave a complex mixture of acidic compounds which was directly esterified with ethereal CH_2N_2.

Chromatographic separation gave, besides the already isolated methyl esters **4b** and **5b**, two more polar compounds which were assigned structures **6b** and **7b** on the basis of their chemical and spectroscopic features.



[1]This paper is No. 6 in a series of studies on aquatic plants distributed in Italy. For paper No. 5 see Monaco and Previtera (1).

Compound **6b** was semi-crystalline, $[\alpha]_D-42°$, with ir hydroxyl absorptions at 3550 and 3490 cm^{-1} that disappeared after acetylation under mild conditions, two carbonyl absorptions at 1750 and 1735 cm^{-1} were attributable to a five member ring ketone and an ester group, respectively, besides an isolated double bond at 1670 cm^{-1}.

Low resolution ms gave a satisfactory fragmentation pattern and hrms (*m/z* 312.1943) gave a molecular formula of $C_{17}H_{28}O_5$. Although the ^{1}H-nmr spectrum was complex, decoupling and nOe experiments allowed most of the signals to be assigned based on the chemical shifts reported for prostaglandin E_1 (5). In particular, irradiation of the H-11 at δ 4.06 caused the H-12α and H-12β multiplets to collapse into two doublets (J=18.2 Hz) centered at δ 2.18 and 2.70, respectively, while the H-10 multiplet at δ 2.36 was simplified. Irradiation of the H-9 proton at δ 5.57 simplified the H-10 methyne and transformed the vinylic H-8 signal at δ 5.66 into a doublet (J=6.9 Hz). The 15.1 Hz coupling between H-8 and H-9 obtained by irradiating the H-7 proton at δ 4.15 was consistent with the *E* configuration at C-8. A nOe effect between H-12 and H-10, as well as the coupling of 11.6 Hz between H-10 and H-14 obtained by irradiation of the H-15 methylene at δ 1.60, also indicated a *trans* configuration of the chains, while a negative Cotton effect in the cd spectrum suggested an α configuration at C-14 (6).

The C-7 configuration may also be tentatively assigned, based on the work of De Clerq *et al.* (7) who assigned the H-13 and H-14 protons of the (15*R*) and (15*S*) series of prostaglandins by chemical shift differences. The similarity in the chemical shift of the H-8 and H-9 protons of **6b** with the corresponding H-14 and H-13 protons of PGE_1 suggest a (7*S*) configuration for **6b**. ^{13}C-nmr data was also consistent with the assigned structure, with carbonyl functions at δ 174.04 and 214.27, doublets due to the OH bearing carbons (C-7 and C-11) at δ 71.82 and 72.92, respectively, and the vinylic carbons at δ 131.59 and 136.52. Compound **7b** was crystalline (mp 77-79°, $[\alpha]_D+12°$) with ir data indicating the presence of hydroxyl groups (3530 and 3430 cm^{-1}), lost after treatment with Ac_2O in dry pyridine, and an ester carbonyl (1735 cm^{-1}). Ms analysis of its trimethylsilyl derivative afforded a parent ion (*m/z* 530) and indicated a molecular formula of $C_{17}H_{30}O_5$, besides a satisfactory fragmentation pattern.

^{1}H-nmr data for **7b** included two vinylic protons (δ 5.57 and 5.46), three OH bearing methynes (δ 4.19, 4.14 and 3.95), a three protons multiplet (δ 2.27), and two methynes (δ 2.16 and 1.77).

The main differences between the ^{1}H-nmr spectra of **6b** and **7b** were the presence of an additional CH-OH group in **7b** and the upfield shifts of the H-12α, H-12β, and vinylic protons. As further evidence of structure $NaBH_4$ reduction of a sample of **6b** was carried out, affording a mixture of triols epimeric at C-13, one of which was identical with **7b** (tlc). A comparison of the chemical shifts of the H-11, H-12α, H-12β, and H-13 proton signals in **7b** and 13 *epi*-**7b** with those of the corresponding signals in PGF_1 and $PGF_{1\beta}$ (8) indicated an α configuration for C-13 in **7b**. In the course of a study of the autoxidative cyclization of lipid hydroperoxides, D.E. O'Connor *et al.* (9) reported the formation of a triol methylester homologous to **7b** by autoxidation of methyl 13-hydroperoxy-9*Z*,11*E*,15*Z*-octadecatrienoate. The ^{13}C-nmr data reported are consistent with those of **7b**.

From a biogenetic point of view the presence of **4a**, **5a**, **6a**, and **7a** appears to be consistent with the existence of two enzymic systems in Lemnaceae. A lipoxygenase-oxidation at C-10 of **4a** would give an 10*R*-hydroperoxy-7*Z*,11*E*,13*Z*-hexadecatrienoic acid intermediate, whose reduction by reductase would afford **5a**, whereas a lipoxygenase oxidation at C-11 would afford an 11*R* peroxy radical, whose cyclization by cyclooxygenase would give a bicycloendoperoxide, a direct precursor of **6a** and **7a**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H-nmr (270 MHz) and ^{13}C-nmr (67.88 MHz) spectra were performed on a Bruker WH 270 FT spectrometer interfaced with an ASPECT 2000 computer. The solvent was $CDCl_3$ and TMS was used as internal standard. Mass spectra were determined with a Kratos MS 30 spectrometer. The plants of *L. minor*, authenticated by professor G. Aliotta, were collected at the Botanical Garden of the University of Naples, where a voucher specimen is deposited.

ISOLATION OF THE ESTERIFIED FATTY ACIDS.—The fresh plants of *L. minor* (1500 g) were homogenized, lyophilized, and then continuously extracted with cold Me_2CO for 2 days. After evaporation in vacuo, the residue (30 g) was dissolved in EtOAc (500 ml) and treated with 2N NaOH (2×200 ml). The aqueous phase was reacidified with 2N H_2SO_4 and extracted with EtOAc (2×200 ml). The organic layer was washed until neutral and evaporated to give a mixture of acidic components (2.5 g) which was directly esterified with ethereal CH_2N_2 and chromatographed on Si gel (75 g, C_6H_6, 450 ml) to afford a mixture (320 mg) of methyl hexadeca-11*Z*-enoate and methyl hexadeca-7*Z*,10*Z*,13*Z*-trienoate [**4b**], which was identified by comparison with authentic material. Elution with C_6H_6-EtOAc (19:1, 300 ml) gave **5b** (36 mg), which was identical to an authentic sample.

DIHYDROXYKETOESTER **6b**.—Elution with C_6H_6-EtOAc (8:2, 450 ml) gave crude **6b** which was purified by preparative tlc (hexane-Et_2O-$CHCl_3$, 55:35:10); (14 mg), $[\alpha]_D$ −42° (*c* 0.5 in EtOH); ir 3550, 3490, 1750, 1735, 1670 cm^{-1}; ms *m/z* 312 $[M]^+$ (2%), 294 $[M\text{-}H_2O]^+$ (11), 279 $[M\text{-}H_2O\text{-}CH_3]^+$ (32), 276 $[M\text{-}2H_2O]^+$ (16), 263 $[M\text{-}H_2O\text{-}OCH_3]^+$ (7), 245 $[M\text{-}2H_2O\text{-}OCH_3]^+$ (4), 193 $[C_{12}H_{17}O_2]^+$ (25), 175 $[C_{12}H_{15}O]^+$ (13), 109 $[C_7H_9O]^+$ (8); ^{1}H nmr δ 5.66 (*m*, 1H), 5.57 (*m*, 1H), 4.15 (*m*, 1H), 4.06 (*m*, 1H), 3.64 (*s*, 3H), 2.70 (*m*, 1H), 2.36 (*m*, 1H), 2.27 (*t*, 2H), 2.18 (*m*, 1H), 1.60 (*m*, 6H), 1.31 (*m*, 4H), 0.91 (*t*, 3H); ^{13}C nmr δ 214.27 (*s*, C-13), 174.04 (*s*, C-1), 136.52 (*d*, C-8), 131.59 (*d*, C-9), 72.97 (*d*, C-7), 71.82 (*d*, C-11), 54.57 (*d*, C-14), 54.25 (*d*, C-10), 45.98 (*t*, C-12), 37.02 (*t*, C-6), 34.00 (*t*, C-2), 29.37 (*t*, C-4), 25.65 (*t*, C-5), 24.78 (*t*, C-3), 21.32 (*t*, C-15), 11.58 (*q*, C-16).

TRIHYDROXYESTER **7b**.—Elution with C_6H_6-EtOAc (1:1, 600 ml) gave crude **7b** (12 mg) which was crystallized from hexane-C_6H_6 (6:4); mp 77-79°, $[\alpha]_D$+12° (*c* 0.6 in EtOH); ir 3530, 3430, 1735 cm^{-1}; ^{1}H nmr δ 5.57 (*m*, 1H), 5.46 (*m*, 1H), 4.19 (*m*, 1H), 4.14 (*m*, 1H), 3.95 (*m*, 1H), 3.66 (*s*, 3H), 2.27 (*m*, 3H), 2.16 (*m*, 1H), 1.77 (*m*, 1H), 1.61 (*m*, 2H), 1.53 (*m*, 1H), 1.45 (*m*, 1H), 1.31 (*br*, 7H), 0.89 (*t*, 3H); ^{13}C nmr δ 174.32 (*s*, C-1), 135.21 (*d*, C-8), 133.17 (*d*, C-9), 78.06 (*d*, C-11), 73.13 (*d*, C-13), 73.11 (*t*, C-6), 56.15 (*d*, C-10), 52.44 (*d*, C-14), 43.15 (*t*, C-12), 37.19 (*t*, C-6), 34.01 (*t*, C-2), 29.37 (*t*, C-4), 25.72, (*t*, C-5), 24.87 (*t*, C-3), 22.86 (*t*, C-15), 13.02 (*q*, C-16).

ACETYLATION OF **6b**.—Pure **6b** (5 mg) was treated with Ac_2O (0.05 ml) and dry pyridine (0.5 ml) overnight. MeOH (1 ml) and toluene (3 ml) were added to the reaction mixture which was evaporated in vacuo to afford a residue (5 mg); ir 1750, 1735, 1660, 1260 cm^{-1}; ^{1}H nmr δ 5.62 (*m*, 1H), 5.51 (*m*, 1H), 5.25 (*m*, 1H), 4.96 (*m*, 1H), 3.65 (*s*, 3H), 3.00 (*m*, 1H), 2.28 (*m*, 2H), 2.14 (*m*, 1H), 2.04 (*s*, 3H), 2.02 (*s*, 3H), 1.60 (*m*, 6H), 1.31 (*m*, 4H), 0.91 (*t*, 3H).

TRIMETHYLSILYL ETHER OF **7b**.—A pure sample of **7b** (2 mg) was treated with (trimethylsilyl)-imidazole (0.1 ml) and pyridine (0.5 ml); ms *m/z* 530 $[M]^+$ (1%), 440 $[M\text{-}(CH_3)_3SiOH]^+$ (3), 324 $[M\text{-}(CH_3)_3SiOH\text{-}(CH_3)_3SiOCHCH_2]^+$ (7), 311 $[M\text{-}(CH_3)_3SiOH\text{-}(CH_2)_5CO_2CH_3]^+$ (6), 285 $[M\text{-}(CH_2)_5CO_2CH_3\text{-}(CH_3)_3SiOCHCH_2]^+$ (12), 195 $[M\text{-}(CH_2)_5CO_2CH_3\text{-}(CH_3)_3SiOCHCH_2\text{-}(CH_3)_3SiOH]^+$ (21), 191 $[(CH_3)_3SiO_2CH]^+$ (8).

ACETYLATION OF **7b**.—A sample of **7b** (6 mg) was esterified with Ac_2O (0.1 ml) in dry pyridine (1 ml) overnight. Work up as for **6b** gave the triacetyl derivative; ^{1}H nmr δ 5.59 (*m*, 1H), 5.22 (*m*, 1H), 4.91 (*m*, 1H), 2.51 (*m*, 2H), 2.08 (*s*, 3H), 2.05 (*s*, 3H), 2.02 (*s*, 3H).

ACKNOWLEDGMENTS

This work was supported by M.P.I. (Ministero della Pubblica Istruzione). The authors are indebted to A. Cantilena, I. Giudicianni, and C. Sepe for their technical assistance. We are grateful to Dr. D.E. O'Connor for providing supplementary material.

LITERATURE CITED

1. P. Monaco and L. Previtera, *Phytochemistry*, **26**, 745 (1987).
2. R.G. Salomon, *Acc. Chem. Res.*, **18**, 294 (1985).
3. J. Rokach and J. Adams, *Acc. Chem. Res.*, **18**, 87 (1985).
4. L. Previtera and P. Monaco, *Phytochemistry*, **22**, 1445 (1983).

5. G. Kotovych and G.H.M. Aarts, *Can. J. Chem.*, **60**, 2617 (1982).
6. M. Miyano and M.A. Stealey, *J. Org. Chem.*, **40**, 1748 (1975).
7. P. DeClercq, M. Samson, D. Tavernier, D. VanHaver, and M. Vandewalle, *J. Org. Chem.*, **42**, 3140 (1977).
8. M. Miyano, C.R. Dorn, and R.A. Mueller, *J. Org. Chem.*, **37**, 1810 (1972).
9. D.E. O'Connor, E.D. Mihelich, and M.C. Coleman, *J. Am. Chem. Soc.*, **106**, 3577 (1984).

Received 24 November 1986