Tetrahedron Vol 47, No 34, pp 7129-7134, 1991
Printed in Great Britain

0040-4020/91 $3 00+ 00

NEW OXYGENATED STEROLS FROM THE WEED *Eichhornia crassipes* SOLMS

Marina Della Greca, Pietro Monaco and Lucio Previtera*

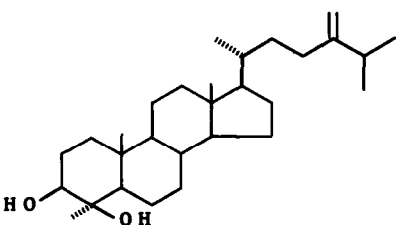
Dipartimento di Chimica Organica & Biologica dell'Università
Via Mezzocannone 16, I-80134 Napoli, Italy

(*Received in UK* 17 *May* 1991)

Key Words *Eichhornia crassipes*; Pontederiaceae, 4-hydroxysterols, plant growth inhibitors

Abstract Three novel sterols **1** - **3** with an uncommon hydroxyl function at C-4 have been isolated from the weed *E crassipes*. The stereostructures of the compounds have been defined on the basis of spectroscopic data. Phytotoxicity test of the sterols revealed valuable biological activity against root seedling growth of radish.

In pursuing our search for allelochemicals from aquatic plants[1], we have reinvestigated *Eichhornia crassipes* Solms, (water hyacinth) a plant whose extracts have been reported to have effects as growth regulators[2]. From the ethyl acetate extract we have isolated three novel sterols, 4α-methyl-5α-ergosta-8,14 24(28)-triene-3β,4β-diol (**1**), 4α-methyl-5α-ergosta-8,24(28)-diene-3β,4β-diol (**2**) and 4α-methyl-5α-ergosta-7,24(28)-diene-3β,4β-diol (**3**) with the unique 4-hydroxy function.



1 Δ8,14 **2** Δ8 **3** Δ7

The sterols, tested for their allelochemical activity, showed effects on the root growth of *Raphanus sativus* L.

Compound **1** showed a molecular ion at m/z 426.3491 in the HRMS spectrum and in the ^{13}C-NMR spectrum exhibited 29 carbons according to a molecular formula $C_{29}H_{46}O_2$. The presence of six olefinic carbons justified three degrees of unsaturation beside the perhydrophenanthrene skeleton A fragment at m/z 301.2173 $[M - C_9H_{17}]^+$ in the MS spectrum suggested a monounsaturated side chain whereas the fragment at m/z 342.2555 $[M - C_6H_{12}]^+$, due to the loss of the terminal part of the side chain, agreed on the presence of a methylene group at C-24 Accordingly, in the ^1H-NMR spectrum the H-28 methylene as two singlets at δ 4.676 and 4.728, the H-25 septet at δ 2.382 and the H-21, H-26 and H-27 methyl doublets at δ 0 968, 1.031 and 1.036 were respectively identifiable These signals were correlated in the H-C COSY to the carbons at δ 105 9, 33 9, 18 8, 21.8 and 21 9. The H-21 chemical shift, compared with the corresponding signals in (20R)- and (20S)-24-methylenelophenol[3], suggested a R configuration at C-20 The remaining unsaturations were univocally located at the 8,14 positions of the steroid nucleus. In fact the UV spectrum showed a strong band at 248 nm, characteristic of heteroannular conjugated dienes, while a DEPT sequence showed three tetrasubstituted carbons at δ 122.4, 141.6, 150.8 and a trisubstituted carbon at δ 117.2 belonging to this moiety. The chemical shifts of the H-15 proton at δ 5.382 and the H-18 methyl at δ 0 821 were in good agreement[4] with these structural features.

The IR spectrum, beside the absorptions due to the already located unsaturations (1640, 1620 cm^{-1}) displayed bands of hydroxyl groups at 3550 and 3380 cm^{-1} and, accordingly, the MS spectrum showed fragments due to the loss of water at m/z 393.3150 $[M - CH_3 - H_2O]^+$ and 375 3059 $[M - CH_3 - 2H_2O]^+$. One of the hydroxyl groups was located at C-3 on the basis of biogenetic considerations. The geminal proton at δ 3.298 appeared as a part X of an ABX system and its couplings were in agreement with a ß-equatorial oriented hydroxyl group. This proton was correlated in the H-C COSY to the methine carbon at δ 75.2 whereas in a long range H-C COSY showed scalar interaction with the carbon at δ 74 1 which, in turn, was correlated to the methyl singlet at δ 1.286. These data were indicative of the presence of geminal methyl and hydroxyl groups at C-4. The 4ß orientation of the hydroxyl function finally resulted from the chemical shift at δ 1.193 of the angular C-19 methyl. In fact, this signal was downfield shifted of about 0.2 ppm respect to the corresponding signals in the 4α methyl sterols series[5] owing to the 1,3 diaxial interaction with the hydroxyl group[6].

The formation of the corresponding acetonide by treatment with acetone

and $CuSO_4$ was a confirm of the molecular structure.

Compound **2** had a molecular formula $C_{29}H_{48}O_2$ according to the MS and ^{13}C-NMR data. ^1H-NMR spectrum showed the H-21, H-26, H-27 methyl doublets at δ 0.964, 1.029, 1.036 and the H-28 methylene singlets at δ 4.664 and 4.720 supporting the same side chain of **1** Accordingly the MS spectrum displayed fragments at m/z 303.2321 $[M - C_8H_{17}]^+$ and 344.2731 $[M - C_6H_{12}]^+$ Beside the C-24 and C-28 carbons at δ 156.7 and 106.0, the ^{13}C-NMR spectrum showed two further tetrasubstituted olefinic carbons at δ 127 6 and 135 0. The corresponding double bond was univocally located at the C-8 position on the basis of the chemical shift at δ 0.621 of the H-18 methyl[7]. Finally the chemical shifts of H-3 , H-19 and H-30 at δ 3.298, 1.177 and 1.282 confirmed the same structural moiety as **1**.

Compound **3** had the same molecular formula $C_{29}H_{46}O_2$. Besides the methyl doublets H-21, H-26, H-27 at δ 0.961, 1.031, 1 037 and the H-28 singlets at δ 4 664 and 4.723, the ^1H-NMR spectrum showed the H-18, H-19 and H-30 methyls at δ 0.541, 1.025 and 1.249, the H-3 double doublet at δ 3.305 and an olefinic proton at δ 5.256 The chemical shifts of the H-18 and H-19 methyls were in agreement with an unsaturation at C-7.

The phytotoxic activity of sterols has been compared with that of coumarin, one of the most potent natural inhibitor of germination and seedling growth. As reported in table 1 sterols **1** and **2** inhibited 40% and 30% of radish root elongation although germination was not affected.

TABLE 1. Effects of Sterols and Coumarin upon Germination and seedling Growth of Radish after four days from soaking.

	Dose (μmol)	germination (%)	root length (mm)
Control	-	100	26
Coumarin	6.0	20	13
1	6 0	100	16
2	6 0	100	18
3	6.0	100	24

According to previous reports[8] the three 4-hydroxysterols may be included in the same biosynthetic pathway a NADPH dependent hydrogenase could convert **1** into **2** which, in turn, could give rise to **3** by isomerase The hydroxyl group at C-4 might be formed during the 4α-demethylation process[9] of the corresponding 4,4 dimethyl sterols by an oxidative

decarbonylation[10] of the formyl intermediates or, alternatively, through a stereospecific hydroxylation of the resulting 4α-methyl sterols by oxygenase[11].

EXPERIMENTAL

UV spectra were obtained on a Perkin-Elmer LAMBDA 7 spectrophotometer. IR spectra were recorded on a Perkin-Elmer mod. 1740 spectrometer. EIMS spectra were performed on a Kratos MS 80 apparatus. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AM 400 spectrometer equipped with a dual probe (H/C 400.135/100.61 MHz) in $CDCl_3$ solutions. One bond and long range H-C COSY experiments were performed with the XHCORR microprogramme.

Isolation of sterols **1 - 3.**

The plants of *E. crassipes* (dry weight 9 Kg) were treated with ethyl acetate to give a crude extract (40 g) which was separated into a neutral and an acid fraction. The neutral material (27 g) was fractionated on Si gel column by step gradient from light petroleum to diethyl ether. The most polar fraction (7.3 g) was further chromatographed on Si gel eluting with light petroleum-ethyl acetate (4 1) to give a mixture of 1 - 3 (150 mg). Preparative argentation tlc (benzene-ethyl acetate 4 1, 2 runs) give pure sterols 1 (50 mg), 2 (15 mg) and 3 (60 mg).

4α-Methyl-5α-ergosta-8,14,24(28)-triene-3β,4β-diol **(1).**

MS. m/z 426.3491, 411.3264, 393.3150, 375.3059, 342.2555, 327.2333, 301.2173, 299.2024. UV λ_{max} (EtOH) 249 nm (ε 21,000). IR ν_{max} ($CHCl_3$) 3550, 3380, 1640, 1620 cm^{-1}. ^{1}H-NMR δ 0.821 (s), 0.968 (d, 6.8 Hz), 1.031 (d, 6.9 Hz), 1.036 (d, 6.9 Hz), 1.193 (s), 1.286 (s), 3.298 (dd, $J_{AX} + J_{BX}$ 18.7 Hz), 4.676 and 4.728 (ss), 5.382 (brs). ^{13}C-NMR δ 36.9 (C-1), 30.8 (C-2), 75.2 (C-3), 74.1 (C-4), 48.5 (C-5), 27.0 (C-6), 27.3 (C-7), 122.4 (C-8), 141.6 (C-9), 34.5 (C-10), 21.3 (C-11), 37.2 (C-12), 44.9 (C-13), 150.8 (C-14), 117.2 (C-15), 35.8 (C-16), 56.9 (C-17), 15.5 (C-18), 17.4 (C-19), 33.7 (C-20), 18.8 (C-21), 35.1 (C-22), 29.6 (C-23), 156.7 (C-24), 33.9 (C-25), 21.8 (C-26), 21.9 (C-27), 105.9 (C-28), 25.2 (C-30).

Acetonide of **1**

Pure 1 (10 mg), dissolved in dry aceton (2 ml), was added of $CuSO_4$ (5 mg) and refluxed 4 hr. After filtration, the solution was evaporated

in vacuo and the residue was chromatographed on preparative Si gel tlc (benzene- ethyl acetate 9 1) to give the corresponding acetonide. ^{1}H-NMR δ 0.838 (s, H-18), 0.965 (d, 6.8 Hz, H-21), 1.033 (d, 6.8 Hz, H-26), 1 039 (d, 6.8, H-27 Hz), 1.188 (s, H-19), 1.390 (s, H-30), 3 895 (dd, H-3), 4 672 and 4.730 (ss, H-28), 5.386 (brs, H-15), 1.449 and 1.524 (ss, Me_2C).

4α-Methyl-5α-ergosta-8,24(28)-diene-3β,4β-diol **(2).**

MS m/z 428.3662, 410.3553, 395.3326, 377.3218, 344 2731, 303.2331, 301.2181 IR ν_{max} 3635, 3540, 1644 cm^{-1}. ^{1}H-NMR δ 0.621 (s), 0.964 (d, 6.6 Hz), 1.029 (d, 6.8 Hz), 1.036 (d, 6.8 Hz), 1.177 (s), 1.282 (s), 3.298 (dd, J_{AX} +J_{BX} 18.4 Hz), 4.664 and 4.723 (ss). ^{13}C-NMR δ 35.6 (C-1), 31.4 (C-2), 76.0 (C-3), 73.9 (C-4), 48.6 (C-5), 22.6 (C-6), 27 8 (C-7), 127.6 (C-8), 135.0 (C-9), 34.0 (C-10), 22.7 (C-11), 36.8 (C-12), 42.3 (C-13), 51.4 (C-14), 23.3 (C-15), 28.7 (C-16), 55.0 (C-17), 11.3 (C-18), 17.1 (C-19), 36.0 (C-20), 18.4 (C-21), 34.2 (C-22), 29.8 (C-23), 156.7 (C-24), 33.4 (C-25), 21.8 (C-26), 21.9 (C-27), 106.0 (C-28), 25.3 (C-30).

Acetonide of **2.**

Acetonide of 2 was prepared as described for **1.** ^{1}H-NMR δ 0.635 (s), 0.966 (d, 6.6 Hz), 1.032 (d, 6.7 Hz), 1.038 (d, 6.7 Hz), 1.171 (s), 1.285 (s), 3.892 (dd), 1.446 and 1.552 (ss, Me_2C).

4α-Methyl-5α-ergosta-7,24(28)-diene-3β,4β-diol **(3).**

MS m/z 428.3651, 410.3542, 395.3301, 377.3199, 344.2719, 326.2856, 303.2342, 301.2160, 283.2069. IR ν_{max} 3620, 3530, 1642. ^{1}H-NMR δ 0.541 (s), 0.961 (d, 6.8 Hz), 1.025 (s), 1.031 (d, 6.9 Hz), 1.037 (d, 6.9 Hz), 1.249 (s), 3.305 (dd, J_{AX} + J_{BX} 18.4 Hz), 4.664 and 4.723 (ss), 5.256 (m). ^{13}C-NMR. δ 37.0 (C-1), 31.0 (C-2), 75.9 (C-3), 73.5 (C-4), 48.3 (C-5), 26.8 (C-6) 117.7 (C-7), 138.9 (C-8), 50.9 (C-9), 36.0 (C-10), 20.9 (C-11), 39.4 (C-12), 43.4 (C-13), 54.9 (C-14), 22.9 (C-15), 27.9 (C-16), 55.9 (C-17), 11.8 (C-18), 14.3 (C-19), 36.2 (C-20), 18.8 (C-21), 34.6 (C-22), 29.7 (C-23), 156.8 (C-24), 33.8 (C-25), 22.0 (C-26), 22.1 (C-27), 105.9 (C-28), 25.5 (C-30).

Acetonide of **3.**

Acetonide of **3** was prepared as described above for **1.** ^{1}H-NMR. δ 0.557 (s), 0.964 (d, 6.8 Hz), 1.033 (d, 6.9 Hz), 1.039 (d, 6.9 Hz), 1.020 (s), 1.352 (s), 1.449 and 1.524 (ss, Me_2C), 3.895 (dd), 5.281 (brs).

Bioassay.

R. sativus L. seeds were germinated on 30 ml layers of Bacto Agar gel (10 g/l H_2O) in covered 9 cm diameter petri dishes previously sterilized and kept at 25 °C with a continuous irradiances of 250 μE m^{-2} sec^{-1}. Experiments with pure coumarin and sterols were carried out by dissolving the substances in acetone (1 mg/20 l) and adsorbing them separately on Whatman filter paper disks (0.6 cm diameter) which were then inserted in the middle of petri dishes after the solvent evaporation. A control containing a filter treated with acetone was included in each determination 25 seeds were inserted in each petri dish after three days in order to allow uniform distribution of sterols and coumarin in agar. Seeds were considered germinated when the protrusion of the embryonic root became evident. Effects on root elongation were determined by measuring to the nearest millimeter the length of the primary root of each seedling four days after soaking.

Acknowledgement. This work has been financially supported from Consiglio Nazionale delle Ricerche (Progetto Finalizzato Chimica Fine II).

REFERENCES

1. Aliotta,G ,Della Greca,M ,Monaco,P.,Pinto,G.,Pollio,A.,Previtera, L *J. Chem. Ecol.* **1990**, *16*, 2637-2646.
2. Issa,M.A. *Can. J. Chem.* .**1988**, *66*, 2777-2781.
3. DellaGreca,M ,Mangoni,L.,Molinaro,A.,Monaco,P.,Previtera,L. *Phytochemistry* **1990**, *29*, 1797-1798.
4. Schmitt,P.,Scheid,F.,Benveniste,P. *Phytochemistry* .**1980**, *19*, 525-530.
5. Akihisa,T.,Yokota,T ,Takahashi,N.,Tamura,T.,Matsumoto,T. *Phytochemistry* **1989**, *28*, 1219-1224.
6. Bhacca,N S.,Williams,D.H. *Application of NMR Spectroscopy in Organic Chemistry*, Holden-DayInc San Francisco, 1974, pp 19-21.
7. Kikuchi,T.,Kadota,S.,Tsubono,K. *Chem. Pharm Bull* **1986**, *34*, 2479-2486.
8. Schmitt,P.,Beneveniste,P. *Phytochemistry* **1979**, *18*, 445-450.
9. Trzaskos,J.M.,Bowen,W D., Shafiee,A.,Fischer,R.T., Gaylor,J.L. *J. Bio. Chem.* **1984**, *259*, 13402-13412.
10 Caputo,R.,Mangoni,L.,Monaco,P.,Previtera,L *Gazz. Chim. Ital.* **1974** *104*, 1269-1278.
11 Stumpf,P.,Conn,E.E. *The Biochemistry of Plants* Academic Press New York, 1981, vol 7 pp 628-633.