Phytochemistry, Vol. 27, No. 3, pp. 887–890, 1988.
Printed in Great Britain.

0031 9422/88 $3.00 + 0.00
Pergamon Journals Ltd.

POLYPRENOLS AND HYDROXYLATED LYCOPERSENES FROM *MYRIOPHYLLUM VERTICILLATUM**

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(*Received* 5 *June* 1987)

Key Word Index—*Myriophyllum verticillatum*; Haloragaceae; terpenoids; polyprenols; hydroxy lycopersene; *iso*-hydroxy lycopersene.

Abstract—In a chemical investigation of the aquatic plant *Myriophyllum verticillatum* three polyprenols and two monohydroxy lycopersenes have been isolated and characterized on the basis of their chemical and physical features. The novel compounds hydroxy lycopersene and *iso*-hydroxy lycopersene may be easily included in the carotenoid biosynthetic pathway.

INTRODUCTION

In pursuing our chemical investigation of aquatic plants distributed in Italy we have now examined *Myriophyllum verticillatum* L., a rather rare species which grows in stagnant and eutrophic water-courses and is able to accumulate heavy metals [1].

RESULTS AND DISCUSSION

The mixture of head-to-tail polyprenols was resolved by means of reversed phase preparative TLC [3] into three pure compounds which were attributed structures **1a**, **1b** and **1c** on the basis of their physical features. The most abundant (**1c**), $C_{65}H_{106}O$ ($[M]^+ = m/z$ 766), showed absorptions at 3565, 3305, 1660 and 1005 cm^{-1} in its IR spectrum. Its ^1H NMR spectrum contained singlet methyl signals at δ1.60, 1.61, 1.68 and 1.74 in a 3:1:9:1 ratio, 24 methylenes between δ2.02 and 2.05, a hydroxymethyl group at δ4.09 as a doublet ($J = 6.7$ Hz), 12 olefinic protons at δ5.12 and an olefinic proton as a triplet ($J = 6.7$ Hz) at δ5.44. These data established the presence of three internal *E* and eight internal *Z* isoprene residues besides the ω and the α-*Z* terminal units. The arrangement of the isomeric units was attributed on the basis of the different ^{13}C NMR shieldings of C-1 and C-3 of isoprene residues in *trans–cis* (δ31.99 and 125.06) *trans–trans* (δ39.73 and 124.30), *cis–cis* (δ32.21 and 125.06) and *cis*-terminal–*cis* (δ29.64 and 125.12) linkages [4].

The other polyprenols showed the same ^1H and ^{13}C NMR signals as **1c**. The relative intensities of the signals as well as MS data were consistent with structures **1a** and **1b**.

Compounds **2a** and **3** could not be separated as free alcohols. The mixture was acetylated with acetic anhydride in dry pyridine under mild conditions to convert **2a** into the corresponding acetate **2b** and then chromatographed to afford pure **2b** and **3**.

Acetoxy lycopersene (**2b**)* had $[\alpha]_D - 4.5°$ and a peak at m/z 604 in its mass spectrum corresponding to a molecular formula $C_{42}H_{68}O_2$. Its IR spectrum showed absorptions at 1736, 1660 and 1240 cm^{-1} indicating the presence of isolated double bonds besides the acetoxyl group. The ^1H NMR spectrum had mehtyl singlets at δ1.61, 1.63, 1.69 and 1.71 in a 6:1:2:1 ratio, two allylic protons coupled to each other at δ2.21 and 2.38, a methine bearing the acetate function at δ5.47 besides eight olefinic protons centred at δ5.11. Irradiation at δ5.47 collapsed the multiplets at δ2.21 and 2.38 into two quartets (the AB part of an ABX system: $J = 13.8$ and 7.3 Hz and $J = 13.8$ and 7.6 Hz respectively) and irradiation of the olefinic signal at δ5.11 simplified the multiplet at δ5.47 and transformed the multiplets at δ2.21 and 2.38 in two quartets (the AB part of an ABX system: $J = 13.8$ and 6.5 Hz and $J = 13.8$ and 6.7 Hz). These data established the isoprenoid nature of **2b** and also suggested the presence of two head-to-head linked isoprene residues with the acetoxyl group at the junction. The MS confirmed the previous hypothesis and indicated a symmetrical carbon skeleton formed from two C_{20} moieties: in fact the spectrum showed fragments at m/z 475, 407, 339, 205, 137 and 69 attributable to the loss of one, two and three isoprene residues through allylic cleavages while the absence of the fragments at m/z 271 and 273, indicated an asymmetrical skeleton.

The ^{13}C NMR spectrum showed only 25 signals which were identified as carbon types through DEPT experiments while an inverse gated decoupling experiment gave their relative intensities. Assignments of individual signals were based either on earlier assigned spectra of analogous isoprenoids [5] or partially through two dimensional heteronuclear chemical shift correlation.

*Part 7 in a series of studies on aquatic plants distributed in Italy. For part 6 see L. Previtera and P. Monaco (1987) *J. Nat. Prod.* (in press).

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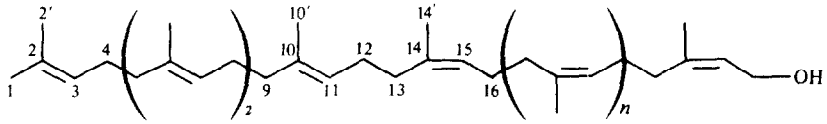
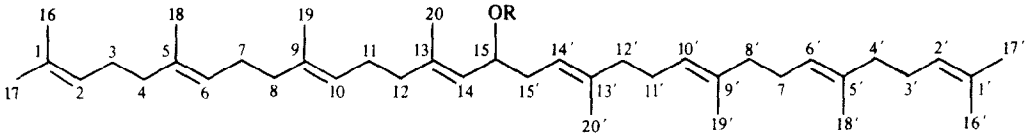
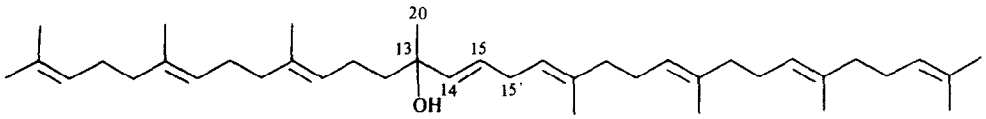
*The numbering system used in this paper for **2a**,**2b** and **3** is according to the IUPAC rule [5]. The IUPAC name of **2b** is 7,8,11,12,15,7′,8′,11′,12′,15′-decahydro-ψ,ψ-caroten-15-ol acetate and that of **3** is 14,15-didehydro-7,8,11,12,13,14,15,7′,8′,11′,12′,15′-ψ,ψ-caroten-13-ol.



1a $n = 5$
1b $n = 6$
1c $n = 7$



2a R = H
2b R = Ac



3

The signal at δ1.61 in the ^{1}H NMR attributed to Me-18, Me-19, Me-18′ and Me-19′ as well as the signal at δ1.63 attributed to Me-20′ were consistent with an *E* configuration at C-5, C-9, C-5′, C-9′ and C-13′ while the configuration at C-13 could not be deduced on the basis of the δ1.71 shift of Me-20. NOE effects between this methyl and the acetate group and the ^{13}C chemical shifts of olefinic carbons C-13 and C-14 at δ140.28 and 118.64 respectively might be indicative of an *E* configuration also at C-13.

Lithium aluminium hydride reduction of **2b** gave **2a**, $[\alpha]_D + 6.2°$ which showed absorptions at 3560, 3300 and 1655 cm^{-1} in its IR spectrum. The ^{1}H NMR spectrum had methyl singlets at δ1.61, 1.65 and 1.69 in a 6:1:3 ratio, a methine proton at δ4.38 and olefinic protons at δ5.12 and 5.21 in a 7:1 ratio. Irradiation at δ5.21simplified the H-15 multiplet while irradiation of the latter transformed the H-14 doublet at δ5.21 ($J = 6.5$ Hz) into a singlet. The mass spectrum showed the molecular ion peak at *m/z* 562 and a fragmentation pattern identical with that of **2b**. The ^{13}C NMR spectrum, compared with that **2b**, showed significant upfield shifts of C-13, C-15 and C-14′ and downfield shifts of C-14 and C-15′ [6]. Finally, the structure **2a** was unequivocally confirmed by conversion into *trans* and *cis* phytoene [7] through dehydratation on neutral alumina.

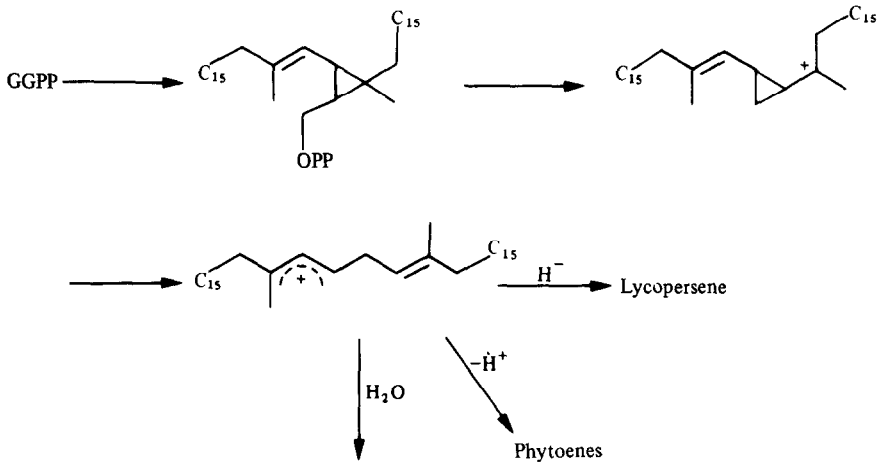
iso-Hydroxy lycopersene (**3**), $[\alpha]_D + 3.5°$, had a molecular formula $C_{40}H_{66}O$ and in its mass spectrum showed relevant peaks at *m/z* 562, 544, 475, 407, 339, 205, 137 and 69. Its IR spectrum was identical with that of **2a**. The ^{1}H NMR spectrum showed a singlet at δ1.26 attributable to a methyl geminal with an hydroxy group, nine vinylic methyls at δ1.61 and 1.69 in a 7:2 ratio and an allylic methylene at δ2.65; the olefinic region showed seven protons centred at δ5.11 and overlapping H-14 and H-15 signals at δ5.58. On addition of Eu(dpm)$_3$ the latter signals were shifted to δ6.26 and 6.10 (1H, *d*, $J = 15.8$ Hz and 1H, *dt*, $J = 6.2$ and 15.8 Hz respectively: the AB part of an ABX_2 system), thus showing an *E* configuration at C-14. The ^{13}C NMR data confirmed the assigned structure with the C-13 quaternary carbon at δ71.74, the C-20 methyl at δ27.69 and the C-14 and C-15 olefinic methines at δ138.85 and 126.31. Dehydration of **3** on neutral alumina gave a mixture of three unsaturated hydrocarbons, one of them being identical with *trans*-phytoene.

From a biogenetical point of view **2a** and **3** may be easily included in the carotenoid pathway: the allylic carbocation intermediate **4**, which rises from prephytoene pyrophosphate and gives lycopersene by addition of hydride and *cis*- and *trans*-phytoene by loss of a proton [8], could add a water molecule at C-13 or C-15 to give **3** and **2a** respectively.

EXPERIMENTAL

^{1}H (270 MHz) and ^{13}C NMR (67.88 MHz): 1D, $CDCl_3$, TMS as internal standard; 2D carbon–proton shift correlations were performed by polarization transfer via J(CH). The number of increments was 256. Polarization transfer delays were set to average one-bond couplings of J(CH) = 140 Hz (the time development for polarization τ_1 was 3.58 msec and the refocusing time for the antiphase signals τ_2 was 1.79 msec). Eight-step phase cycling was used for quadrature detection and N-type selection.

Exponential multiplication in the ^{13}C domain and Gaussian resolution enhancement in the ^{1}H domain were applied to 256 × 1024 data matrices prior to Fourier transformation. The



2a and **3**

inverse gated experiment was carried out with a delay 20 sec (~ 5 T1). *M. verticillatum* collected from an irrigation canal near Naples was identified by Prof. G. Aliotta.

Isolation of polyprenoids. Fresh plants of *M. verticillatum* (2 Kg) were homogenized and lyophilized to afford material which was extracted with cold Et_2O. The ethereal extract was treated with 2 N NaOH, neutralized with 2 N H_2SO_4 and evaporated to give a residue (500 mg) which was chromatographed on a column of silica gel. Petrol–Et_2O (49:1, 300 ml) eluted acetyl phytol (250 mg) and (4:1, 400 ml) eluted phytol (120 mg). The mixture eluted with petrol–Et_2O (19:1 300 ml, 30 mg) was dissolved in dry pyridine (1.0 ml) and treated with Ac_2O (0.5 ml) overnight. MeOH and toluene were added and the soln was evaporated *in vacuo*. Prep TLC (hexane–Et_2O 19:1) gave pure acetoxy lycopersene (**2b**) (18 mg) and *iso*-hydroxy lycopersene (**3**) (7 mg).

2b had $[\alpha]_D$ −4.5° ($CHCl_3$; *c* 0.9); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1736, 1660 and 1240; MS *m/z* (rel. int.): 604 (3), 544 (23), 475 (47), 407 (52), 339 (41), 205 (25), 137 (20), 69 (100); ^1H NMR: δ1.61 (*s*, 18H), 1.63 (*s*, 3H), 1.69 (*s*, 6H), 1.71 (*s*, 3H), 2.02 (*s*, 3H), 2.21 (*m*, 1H), 2.38 (*m*, 1H), 5.11 (*m*, 8H), 5.49 (*m*, 1H); ^{13}C NMR: δ15.96 (C-18, C-19, C-18′, C-19′), 16.27 (C-20′), 17.65 (C-16, C-16′), 25.68 (C-17, C-17′), 26.25 (C-11), 26.62 (C-7, C-7′, C-11′), 26.71 (C-3, C-3′), 33.53 (C-15′), 39.68 (C-4, C-8, C-4′, C-8′), 39.79 (C-12, C-12′), 71.31 (C-15), 118.64 (C-14), 123.15 (C-14′), 123.67 (C-10), 124.03 (C-10′), 124.14 (C-6, C-6′), 124.33 (C-2, C-2′), 131.16 (C-1, C-1′), 134.86 (C-5, C-5′), 135.02 (C-9′), 135.26 (C-9), 138.03 (C-13′), 140.28 (C-13), 21.31 and 170.34 (CH_3CO).

A pure sample of **2b** (8 mg) in dry Et_2O (2 ml) was treated with $LiAlH_4$ (excess) to give pure **2a** (7 mg), $[\alpha]_D$ +6.2° (*c* 0.7 in $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3560, 3300, 1655; MS *m/z* (rel. int): 562 (5), 544 (42), 475 (40), 407 (38), 339 (28), 205 (28), 137 (25), 69 (100); ^1H NMR: δ1.61 (*s*, 18H), 1.65 (*s*, 3H), 1.69 (*s*, 9H), 4.38 (*m*, 1H), 5.12 (*m*, 7H), 5.21 (*d*, 1H, *J* = 6.5 Hz); ^{13}C NMR: δ16.31 (C-20′), 16.68 (C-20), 26.37 (C-11), 36.43 (C-15′), 39.59 (C-12), 39.88 (C-12′), 68.40 (C-15), 119.68 (C-14′), 123.83 (C-14), 138.31 (C-13′), 138.59 (C-13).

Dehydration of **2a**. A pure sample of **2a** (7 mg) was absorbed on neutral Al_2O_3 (grade I) and eluted after 3 hr with petrol–Et_2O. *cis*-Phytoene (49:1, 4 mg) and *trans*-phytoene (24:1, 2 mg) had UV and ^1H NMR spectra identical with those described in ref. [7].

3 had $[\alpha]_D$ +3.5° ($CHCl_3$; *c* 0.5); IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3550, 3305, 1660; MS *m/z* (rel. int.): 562 (3), 544 (35), 475 (35), 407 (30), 339 (32), 271 (60), 205 (33), 137 (40), 69 (100); ^1H NMR: δ1.26 (*s*, 3H), 1.61 (*s*, 21H), 1.69 (*s*, 6H) 5.11 (*m*, 7H), 5.58 (*m*, 2H) [Eu(dpm)$_3$: 6.10 (*dt*, 1H, *J* = 6.2 and 15.8 Hz), 6.26 (*d*, 1H, *J* = 15.8 Hz); ^{13}C NMR: δ15.85 (C-19), 15.96 (C-18, C-18′, C-19′), 16.21 (C-20′), 17.65 (C-16, C-16′), 19.12 (C-11), 25.67 (C-17, C-17′), 26.62 (C-7, C-7′, C-11′), 26.73 (C-3, C-3′), 27.69 (C-20), 29.67 (C-15′), 39.68 (C-4, C-8, C-4′, C-8′, C-12′), 39.88 (C-12), 71.74 (C-13), 124.14 (C-6, C-6′, C-10′), 124.37 (C-2, C-2′), 124.62 (C-10), 126.31 (C-15), 128.80 (C-14′), 131.16 (C-1, C-1′), 132.35 (C-13′), 134.02 (C-9), 134.86 (C-5, C-5′ C-9′), 138.85 (C-14). Dehydration of **3** on neutral Al_2O_3 gave mainly a polyene which had the same R_f as *trans*-phytoene on TLC.

Polyprenols (**1**). The mixture eluted with petrol–Et_2O (9:1) (40 mg) was separated by reversed phase prep TLC (C-18, Me_2CO–H_2O 9:1) into three pure compounds: [3*E*,7*Z*]-11-prenol (**1a**): 10 mg, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3575, 3305, 1660, 1005, MS *m/z* (rel. int.): 766 (4), 748 (10), 679 (4), 611 (4), 543 (3), 475 (6), 407 (6), 339 (4), 271 (4), 203 (10), 135 (25), 121 (35), 95 (50), 81 (70), 69 (100); ^1H NMR: δ1.62 (*s*, 12H), 1.65 (*s*, 21H), 1.75 (*s*, 3H), 2.02 and 2.05 (*ss*, 40H), 4.08 (*d*, 2H, *J* = 6.7 Hz), 5.09 (*m*, 10H), 5.42 (*t*, 1H, *J* = 6.7 Hz); ^{13}C NMR: δ15.97 (C-6′, C-10′), 17.65 (C-2′), 23.38 (C-18′, C-22′, C-26′, C-30′, C-34′, C-38′, C-42′), 25.61 (C-1), 26.37 (C-16, C-20, C-24, C-28, C-32, C-36, C-40), 26.64 (C-4, C-8, C-12), 29.64 (C-41), 31.99 (C-17), 32.21 (C-21, C-25, C-29, C-33, C-37), 39.73 (C-5, C-9, C-13), 59.02 (C-44), 124.19 (C-15), 124.30 (C-7, C-11), 124.57 (C-3), 125.06 (C-19, C-23, C-27, C-31, C-35, C-39), 125.12 (C-43), 131.28 (C-2), 134.85 (C-18), 135.25 (C-6, C-10), 135.42 (C-22, C-26, C-30, C-34, C-38), 136.18 (C-14), 139.70 (C-42). [3*E*,8*Z*]-12-prenol (**1b**): 8 mg, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3575, 3305, 1660, 1005; MS: *m/z* (rel. int.): 834 (3), 816 (9), 747 (5), 679 (4), 611 (4), 543 (3), 475 (8), 407 (5), 339 (5), 271 (7), 203 (10), 135 (25), 121 (38), 95 (46), 81 (75), 69 (100); ^1H NMR: δ1.62 (*s*, 12H), 1.65 (*s*, 24H), 1.75 (*s*, 3H), 2.02 and 2.05 (*ss*, 44H), 4.09 (*d*, 2H), 5.09 (*m*, 11H), 5.43 (*t*, 1H); ^{13}C NMR; δ15.97 (C-6′, C-10′, C-14′), 17.65 (C-2′), 23.38 (C-18′, C-22′, C-26′, C-30′, C-34′, C-38′, C-42′, C-46′), 25.61 (C-1), 26.37 (C-16, C-20, C-24, C-28, C-32, C-36, C-40, C-44), 26.64 (C-4, C-8, C-12), 29.64 (C-45), 31.99 (C-17), 32.21 (C-21, C-25, C-29, C-33, C-37, C-41), 39.73 (C-5, C-9, C-13), 59.02 (C-48), 124.19 (C-15), 124.30 (C-7, C-11), 124.57 (C-3), 125.06 (C-19, C-23, C-27, C-31, C-35, C-39, C-43), 125.12 (C-47), 131.28 (C-2), 134.85 (C-18), 135.25 (C-6, C-10), 135.42 (C-22, C-26, C-30, C-34, C-38, C-42), 136.18 (C-14), 139.70 (C-46). [3*E*,9*Z*]-13-prenol (**1c**): 17 mg; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3575, 3305, 1660, 1005 cm^{-1}; MS *m/z* (rel.

int.): 902 (4), 884 (10), 815 (6), 747 (4), 679 (4), 611 (6), 543 (5), 475 (10), 407 (5), 339 (7), 271 (7), 203 (10), 135 (30), 121 (38), 95 (50), 81 (75), 69 (100); ^{1}H NMR: δ1.62 (*s*, 12H), 1.65 (*s*, 27H), 1.75 (*s*, 3H), 2.02 and 2.05 (*ss*, 48H), 4.09 (*d*, 2H), 5.09 (*m*, 12H), 5.43 (*t*, 1H); ^{13}C NMR δ15.97 (C-6′, C-10′, C-14′), 17.65 (C-2′), 23.38 (C-18′, C-22′, C-26′, C-30′, C-34′, C-38′, C-42′, C-46′), 25.61 (C-1), 26.37 (C-16, C-20, C-24, C-28, C-32, C-36, C-40, C-44, C-48), 26.64 (C-4, C-8, C-12), 29.64 (C-49), 31.99 (C-17), 32.21 (C-21, C-25, C-29, C-33, C-37, C-41, C-45), 39.73 (C-5, C-9, C-13), 59.02 (C-52), 124.19 (C-15), 124.30 (C-7, C-11), 124.57 (C-3), 125.06 (C-19, C-23, C-27, C-31, C-35, C-39, C-43, C-47), 125.12 (C-51), 131.38 (C-2), 134.85 (C-18), 135.25 (C-6, C-10), 135.42 (C-22, C-26, C-30, C-34, C-38, C-42, C-46), 136.18 (C-14), 139.70 (C-50).

Acknowledgement—This work was supported by M.P.I. (Ministero della Pubblica Istruzione).

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