Nonpolar Components of the Latex of *Euphorbia peplus*

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The less polar fractions of the latex of *Euphorbia peplus* were found to contain obtusifoliol, cycloartenol, 24-methylenecycloartanol, lanosterol, and 24-methylenelanosterol in the free and esterified triterpene alcohol fractions; 9-*cis*-tricosene as the major component of the hydrocarbon fraction; and a new acyclic triterpene alcohol named peplusol (**1**). The structure of **1** was determined as the *R*-isomer of (all-*E*)-2-(5,9-dimethyl-1-methylene-4,8-decadienyl)-5,9,13-trimethyl-4,8,12-tetradecatrien-1-ol by spectral and chemical methods.

Plants in the family Euphorbiaceae are well-known for the chemical diversity of their isoprenoid constituents.[1] Biologically active diterpenoids have been the focus of many phytochemical studies,[2,3] while the triterpene alcohols found in the latex of *Euphorbia* species have been used as chemotaxonomic markers.[4,5] In connection with our interest in the biosynthetic origins of chemical diversity in triterpenoids, we have carried out an analysis of the diminutive species *Euphorbia peplus* L. (Euphorbiaceae).

This species of *Euphorbia* is native to Europe, but now grows as a weed throughout the world.[6,7] There are numerous documented ethnopharmaceutical uses of this plant,[8] many of which are thought to be due to its proinflammatory diterpenoid constituents.[6,7,9−11] The latex of *E. peplus* has been reported to contain lanosterol, the triterpene alcohol precursor of sterols in animals and fungi.[4] Lanosterol is uncommon in plants, which utilize cycloartenol for sterol biosynthesis. Subsequent reports of triterpene alcohols from this plant have confirmed the presence of lanosterol among a variety other triterpene alcohols.[12,13] Whole plant material has been employed in these studies, perhaps due to the difficulty of collecting the latex of such a small plant. The epicuticular waxes, on the other hand, are reported to contain only pentacyclic triterpene alcohols.[14]

The technical problem of collecting the latex of *E. peplus* was solved by using filter paper to absorb the droplets released when the plant stem is broken. Fractionation on Si gel provided a hydrocarbon fraction, triterpene esters, free triterpene alcohols, and 20-deoxyingenol 3-angelate, as well as a more polar diterpenoid fraction, which was not further investigated. The identity of 20-deoxyingenol 3-angelate was determined by comparison of its ^{1}H and ^{13}C NMR spectra with published data.[9,10] Separation of the triterpene alcohols by reversed-phase HPLC and ^{1}H NMR analysis showed that these consisted entirely of tetracyclic triterpene alcohols of the lanostane family, namely, cycloartenol, lanosterol, their respective 24-methylene derivatives, and obtusifoliol (Table 1). The triterpene alcohol composition of *E. peplus* is therefore similar to *E. lathyris* in containing both cycloartenol and lanosterol.[15] In the latter species we have shown that lanosterol is produced by direct cyclization of squalene oxide rather than through ring-opening of cycloartenol.[15] Unlike *E. lathyris*, which contains significant amounts of butyrospermol, we found no nonlanostane triterpene alcohols in *E. peplus* latex.

Table 1. Triterpene Alcohols of *Euphorbia peplus* Latex

triterpene alcohol	HPLC t_R (min)	free alcohols (%)	triterpene esters (%)
obtusifoloiol	47.0	2	0
lanosterol	51.0	23	17
24-methylenelanosterol	55.2	2	3
cycloartenol	56.4	44	66
24-methylenecycloartanol	59.8	29	14

A new noncyclic triterpene alcohol (**1**) was found to comprise approximately 7% of the ethyl acetate-soluble fraction of the latex of *E. peplus*. Similarity of the ^{1}H NMR spectrum to that of squalene implied that the molecule consisted largely of regular isoprenoid chains. The presence of five olefinic proton signals clustering at 5.1 ppm and seven allylic methyl groups indicated that there were five regular isoprene units in the chains, two of which were terminal. The appearance of two broad singlets at 4.96 and 4.87 ppm was indicative of an olefinic methylene, and a multiplet of two protons at 3.55 ppm suggested a primary alcohol. The ^{13}C NMR spectrum showed 30 carbon signals, consistent with assignment as a triterpene, and a molecular ion at *m*/*z* 426 in the mass spectrum indicated an elemental formula of $C_{30}H_{50}O$, which was confirmed by HRFABMS. The complete structure of the molecule was determined by a combination of the COSY, HMQC, and HMBC techniques to be (all-*E*)-2-(5,9-dimethyl-1-methylene-4,8-decadienyl)-5,9,13-trimethyl-4,8,12-tetradecatrien-1-ol (Figure 1). The new triterpene alcohol, named peplusol (**1**), represents a biosynthetic variant of the type of head-to-head condensation of two farnesyl units that generally leads to squalene.[16] It is similar in this respect to lavandulol, a monoterpene found in lavender oil.[17] Assignment of the chirality of **1** was made by preparing the (*R*)- and (*S*)-α-methoxyphenylacetic acid (MPA) esters.[18] Differences in the ^{1}H NMR shifts of the 15′-methyl and the (*Z*)-15-hydrogen (assigned by ROESY) indicates an *R*-configuration ($\Delta\delta^{RS} = +0.02$ and -0.03 ppm, respectively). This assignment is based on a preference for the *sp-a/a* conformer,[18] which was determined by computer modeling (MMFF). Additional evidence supporting the *R*-configuration was provided by the negative value of the optical rotation (α_D −18.0°), which is also found for the monoterpenoid analogue (*R*)-lavandulol (α_D −10.05°).[19] The *R*-configuration is also found in presqualene alcohol.[20] The racemic form of peplusol (**1**) has been

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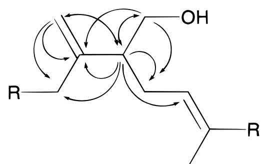
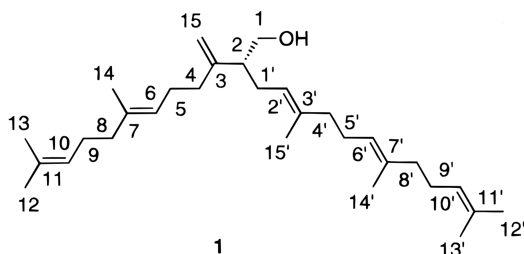


Figure 1. HMBC correlations (^{1}H → ^{13}C) for peplusol (**1**).

prepared as a synthetic intermediate in a study of squalene biosynthesis. The reported spectral data are consistent with our own.[21]



The hydrocarbon fraction of *E. peplus* latex appeared to contain primarily a single long-chain olefinic compound by ^{1}H NMR. GC–MS showed this compound to be a monounsaturated C_{23} hydrocarbon and to represent 80% of the hydrocarbon fraction. Monounsaturated C_{21}, C_{25}, and C_{27} hydrocarbons each represented about 5% of the mixture, together with much smaller amounts of the saturated compounds. The ^{1}H NMR spectrum of this hydrocarbon fraction indicated that it was mainly an isomer of *cis*-tricosene bearing an internal double bond. The location of the double bond was determined through the formation of a dimethyl disulfide (DMDS) adduct.[22,23] Mass spectral analysis of the DMDS adduct showed two strong peaks at *m/z* 173 and *m/z* 243, situating the double bond at the C9 position. The *E. peplus* hydrocarbon is therefore 9-*cis*-tricosene, the sexual pheromone of the housefly.[24] Comparison of the natural hydrocarbon with commercially available 9-*cis*-tricosene (muscalure) and their DMDS adducts with each other showed that these were identical.

Experimental Section

General Experimental Procedures. The optical rotation was measured using a JASCO DIP-1000 polarimeter. UV data were obtained using a Perkin–Elmer Lambda 40 spectrometer. IR spectra were recorded using a Perkin–Elmer Paragon 1000 FT-IR. NMR spectra were acquired using Bruker Avance-300 and Bruker Avance-600 instruments; using $CDCl_3$ as the solvent and referenced to residual $CHCl_3$ signals (^{1}H, 7.262 ppm; ^{13}C, 77.00 ppm). GC–MS data were obtained using a Hewlettn-Packard 5890 series II gas chromatograph with a Hewlett-Packard 5989B mass spectrometer. Electrospray MS data were obtained with a JEOL JMS-LC Mate LCMS-system. The HRMS was measured with a JEOL JMS-HX110HF mass spectrometer. TLC was performed on Whatman aluminum-backed plates coated with a 0.25-mm layer of Si gel 60 F_{254}. Column chromatography was carried out on Si gel 60 (170–400 mesh; Fisher Scientific). Reversed-phase HPLC was carried out using a Waters 6000A pump, Waters R401 differential refractometer, and two Altex Ultrasphere ODS 5-μm 10 × 250 mm columns in series, at a flow rate of 3 mL/min MeOH.

Plant Material. The latex of *Euphorbia peplus* was collected in late June of 1998 and 1999 from plants (ca. 20 cm tall) growing as weeds in the garden of J.-L. G. in Syracuse, NY. Latex was collected by breaking the tops of the plants and absorbing the droplets of latex with preweighed filter paper. The plant was identified by Prof. Dudley Raynal (Biology Department, SUNY–ESF), and voucher specimens have been deposited in the herbarium at SUNY–ESF (SYRF).

Extraction and Isolation. The latex (6.9 g) was extracted with ethyl acetate (15 mL) at room temperature overnight. Filtration and evaporation gave 490 mg of an oily residue that was fractionated by Si gel chromatography using a gradient of 0% to 33% ethyl acetate in hexane. Six fractions were obtained (TLC R_f values measured using hexane–EtOAc 4:1): a hydrocarbon fraction containing mainly 9-*cis*-tricosene (7.8 mg, R_f 1.0), triterpenyl fatty esters (25.6 mg, R_f 0.94), peplusol (**1**) (33.2 mg, R_f 0.56), free triterpene alcohols (136.3 mg, R_f 0.31), 20-deoxyingenol 3-angelate (30.2 mg, R_f 0.25),[9,10] and a mixture of more polar diterpenoids (61.4 mg) that was not investigated further. Latex collected from senescent plants in late August showed a similar composition.

Separation of the tetracyclic triterpene alcohols was carried out by reversed-phase HPLC. The triterpene fatty esters were saponified with 10% KOH–EtOH at reflux prior to analysis. The triterpene alcohols were identified by comparison of their ^{1}H NMR spectra (300 MHz) with those of authentic samples.

Peplusol (1): $[\alpha]^{25}_D$ −18.0° (*c* 0.74, *i*-PrOH); UV no absorbance over 215 nm; IR (film) ν_{max} 3356, 2966, 2922, 1445, 1378 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.14–5.07 (5H, m, H-6, H-10, H-2′, H6′, H-10′), 4.96 (1H, s, H-15), 4.87 (1H, s, H-15), 3.57 (1H, dd, J = 10.9, 5.2 Hz, H-1), 3.54 (1H, dd, J = 10.7, 7.0 Hz, H-1), 2.28 (1H, ddt, J = 7.2, 5.5, 7.2 Hz, H-2), 2.19–2.12 (3H, m, 2H-5, H-1′), 2.12–2.01 (9H, m, 2H-4, 2H-5, H-1′, H-5′, H-9′), 2.01–1.95 (6H, m, 2H-8, 2H-4′, 2H-8′), 1.68 (6H, s, H-13, H-13′), 1.61 (6H, s, H-12, H-12′), 1.60 (6H, s, H-14′, H-14 or H-15′), 1.59 (3H, s, H-14 or H-15′); ^{13}C NMR (150 MHz, $CDCl_3$) δ 149.5 (C-3), 136.4, 135.5, 135.0, 131.3, 131.2 (C-7, C-11, C-3′, C-7′, C-11′), 124.3, 124.2, 124.1, 123.8 (C-6, C-10, C-6′, C-10′), 122.1 (C-2′), 110.9 (C-15), 63.9 (C-1), 48.7 (C-2), 39.7, 39.7, 39.6 (C-8, C-4′, C-8′), 34.3 (C-4), 29.0 (C-1′), 26.7, 26.6, 26.5 (C-5, C-9, C-9′), 26.2 (C-5), 25.6, 25.6 (C-13, C-13′), 17.6 (C-12 and C-12′), 16.1, 16.0, 15.9 (C-14, C-14′, C-15′); EIMS *m/z* 426 (M^+, $C_{30}H_{50}O$, 9), 395 (6), 357 (4), 289 (3), 259 (4), 123 (7), 121 (11), 109 (11), 107 (12), 95 (13), 93 (13), 81 (25), 69 (100); HRFABMS *m/z* 427.3947 $[M + H]^+$; calcd for $C_{30}H_{51}O$ 427.3940.

Methoxyphenylacetic Acid Esters of 1. Peplusol (**1**, 3 mg), methoxyphenylacetic acid (4 mg), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (5 mg), and 4-(dimethylamino)pyridine (0.5 mg) were dissolved in 0.5 mL CH_2Cl_2.[25] After 4 h at room temperature, purification by Si gel TLC (hexane–EtOAc 9:1) gave the product in quantitative yield.

(*R*)-MPA ester: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.42 (2H, d, J = 7.7 Hz, Ph), 7.36–7.30 (3H, m, Ph), 5.11–5.05 (4H, m, H-6, H-6′, H-10, H-10′), 5.01 (1H, t, J = 7.1 Hz, H-2′), 4.76 [1H, s, (*E*)-H-15], 4.73 (1H, s, *CH*OMe), 4.65 [1H, s, (*Z*)-H-15], 4.12 (1H, dd, J = 10.7, 7.1 Hz, H-1), 4.06 (1H, dd, J = 10.7, 6.6 Hz, H-1), 3.40 (3H, OMe), 2.31 (1H, quint, J = 7.1 Hz, H-2), 2.11–2.00 (10 H, m, H-5, H-5′, H-9, H-9′), 2.00–1.90 (8H, m, H-4, H-4′, H-8, H-8′), 1.68 (6H, s, H-13, H-13′), 1.60 (3H, s, H-12, H-12′, H-14 or H-14′), 1.60 (3H, s, H-12, H-12′, H-14 or H-14′), 1.60 (3H, s, H-12, H-12′, H-14 or H-14′), 1.58 (3H, s, H-14 or H-14′), 1.51 (3H, s, C-15′); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.6 (s), 148.4 (s, C-3), 136.7 (s), 136.3 (s), 135.2 (s), 135.0 (s), 131.3 (s), 131.2 (s), 128.6 (d), 128.5 (2C, d), 127.3 (2C, d), 124.4 (d), 124.3 (d), 124.1 (d), 123.9 (d), 121.4 (d, C-2′), 110.7 (t, C-15), 82.6 (d, *CH*OMe), 66.8 (t, C-1), 57.3 (q, OMe), 44.9 (d, C-2), 39.7 (t), 39.7 (t), 39.7 (t), 34.6 (t, C-4), 28.9 (t, C-1′), 26.8 (t), 26.7 (t), 26.6 (t), 26.2 (t), 25.7 (2C, q), 17.7 (2C, q), 16.1 (q), 16.0 (q), 16.0 (q); positive ion ESIMS, *m/z* 597.0 $[M + Na]^+$.

(*S*)-MPA ester: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.42 (2H, d, J = 7.7 Hz, Ph), 7.36–7.30 (3H, m, Ph), 5.11–5.05 (4H, m, H-6, H-6′, H-10, H-10′), 5.00 (1H, t, J = 7.1 Hz, H-2′), 4.76 [1H, s, (*E*)-H-15], 4.73 (1H, s, *CH*OMe), 4.68 [1H, s, (*Z*)-H-15], 4.11 (1H, dd, J = 11.0, 7.1 Hz, H-1), 4.07 (1H, dd, J = 11.0, 6.3 Hz, H-1), 3.41 (3H, OMe), 2.32 (1H, quint, J = 6.9 Hz, H-2), 2.09–2.00 (10 H, m, H-5, H-5′, H-9, H-9′), 2.00–1.93 (6H, m, H-4′, H-8, H-8′), 1.93–1.88 (2H, m, H-4), 1.68 (6H, s, H-13, H-13′), 1.60 (6H, s, H-12, H-12′), 1.59 (3H, s, H-14, H-14′), 1.58

(3H, s, H-14, H-14′), 1.49 (3H, s, H-15′); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.6 (s), 148.5 (s, C-3), 136.7 (s), 136.3 (s), 135.2 (s), 135.0 (s), 131.3 (s), 131.3 (s), 128.6 (d), 128.5 (2C, d), 127.2 (2C, d), 124.4 (d), 124.3 (d), 124.1 (d), 123.9 (d), 121.5 (d, C-2′), 110.6 (t, C-15), 82.6 (d, *CH*OMe), 66.7 (t, C-1), 57.4 (q, OMe), 45.0 (d, C-2), 39.7 (t), 39.7 (t), 39.7 (t), 34.5 (t, C-4), 28.9 (t, C-1′), 26.8 (t), 26.7 (t), 26.6 (t), 26.2 (t), 25.7 (2C, q), 17.7 (2C, q), 16.1 (q), 16.0 (q), 16.0 (q); positive ion ESIMS, *m/z* 574.9 $[M + H]^+$, 596.9, $[M + Na]^+$.

Dimethyl Disulfide Adduct Formation.[22,23] A portion of the hydrocarbon fraction (ca. 1 mg) in 0.5 mL hexane was treated with 50 μL DMDS and 0.5 mL of a 6% solution of iodine in ether. After 20 h at room temperature, the sample was partitioned between saturated aqueous sodium thiosulfate and hexane−EtOAc (4:1). The organic layer was filtered through Si gel. Mass spectrometry and ^{1}H NMR showed complete conversion to the DMDS adduct of 9-*cis*-tricosene.

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