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ARACHISPRENOLS: POLYPRENOLS POSSESSING A GERANYL RESIDUE FROM *ARACHIS HYPOGAEA*

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Key Word Index—*Arachis hypogaea*; Leguminosae; peanut; leaves; polyprenols; arachisprenol; glycinoprenol; ficaprenol.

Abstract—Three novel polyprenols, named arachisprenols 10–12, in addition to four glycinoprenols and five ficaprenols were isolated from the leaves of *Arachis hypogaea*. The arachisprenols comprised a geranyl residue, seven to nine interval (*Z*)-prenyl residues and a *Z* α-terminal residue aligned in that order on the basis of their IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. © Elsevier Science Ltd

INTRODUCTION

Polyprenols comprising (*E*)- and (*Z*)-prenyl residues occur widely in animals, microorganisms and higher plants, and can be classified into four groups: betulaprenol-polyprenols, ficaprenol-polyprenols, glycinoprenol-polyprenols and other polyprenols. Betulaprenol-polyprenols have a farnesyl residue [1–5], ficaprenol-polyprenols have a geranylgeranyl residue [6–11] and glycinoprenol-polyprenols have a phytyl residue [12, 13] at each ω-end of their prenyl chains, respectively. Other polyprenols contain one or more saturated prenyl residues at the α-terminal residue and/or the ω-end of their prenyl chain; dolichols [14–17] possess one saturated α-terminal residue and hexahydropolyprenols [18] possess one saturated α-terminal residue and a more saturated prenyl residue at the ω-end of their prenyl chains. Recently, the extensive work on the distribution of ficaprenol-polyprenols and dolichols in plants was performed by Chojnacki's group [19–24].

In the course of the structural elucidation of polyprenols from higher plants, we investigated the chemical constituents in leaves of peanuts (*Arachis hypogaea*) and found the presence of 12 polyprenols, which could be classified into three homologous series. The first contained three novel polyprenols possessing a geranyl residue at the ω-end of each prenyl chain, named arachisprenols 10–12, the second, four glycinoprenols, glycinoprenols 7–10, of which glycinoprenols are new compounds, and the third, five ficaprenols, ficaprenols 8–12. This is the first report on polyprenols having a geranyl residue.

RESULTS AND DISCUSSION

The hexane-soluble fraction of a methanol extract of peanut leaves was subjected to silica gel CC to give a polyprenol fraction. This was analyzed by reverse-phase HPLC and contained 12 polyprenols (Fig. 1). It was purified repeatedly by reverse-phase HPLC to give compounds **1**–**12**, which are numbered in order of increasing retention time. Compounds **1**, **3**, **6**, **9** and **12** and compounds **7** and **10** were identified as ficaprenols 8–12 and glycinoprenols 9 and 10, respectively, by direct comparisons of their chromatographic behaviour on reverse-phase HPLC and their IR, ^{1}H NMR, ^{13}C NMR and mass spectra with those of authentic samples [7–11, 13]. Compounds **2** and **4** were suggested to be glycinoprenol-type polyprenols on the basis of their chromatographic and spectroscopic properties and then named glycinoprenols 7 and 8, respectively. New compounds **5**, **8** and **11** were named arachisprenols 10–12, respectively.

Arachisprenol 10 (**5**) has a molecular formula of $C_{50}H_{82}O$ on the basis of its $[M]^+$ at *m/z* 698.6304 and a dehydrated ion peak at *m/z* 680.6254 ($[C_{50}H_{80}]^+$) in the high-resolution EI-mass spectrum. The ^{1}H NMR spectrum indicated the presence of olefinic protons [δ 5.44 (1H, *br t*, *J* = 7.3 Hz) and 5.13 (9H, *m*)] and a hydroxylated methylene [δ 4.10 (2H, *br d*, *J* = 7.3 Hz)]. The signal at δ 1.60 (6H, *br s*) was assigned to the methyl protons of an internal (*E*)-prenyl residue (5*E*) and an ω-end prenyl residue (5ω). The signal at δ 1.68 (24H, *br s*) was assigned to the methyl protons

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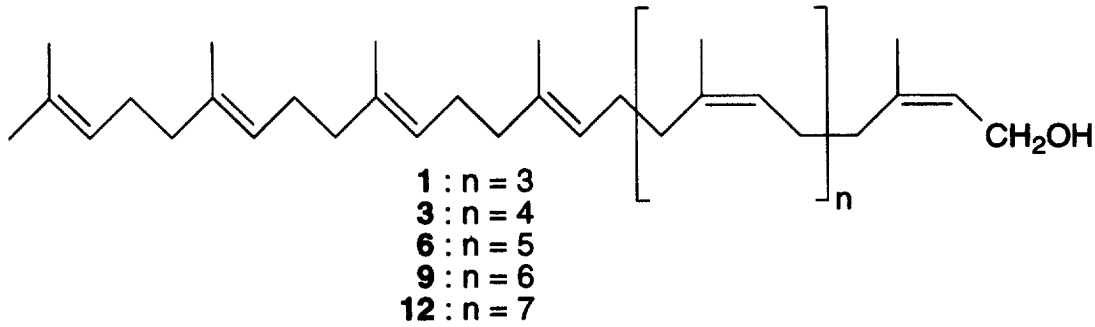
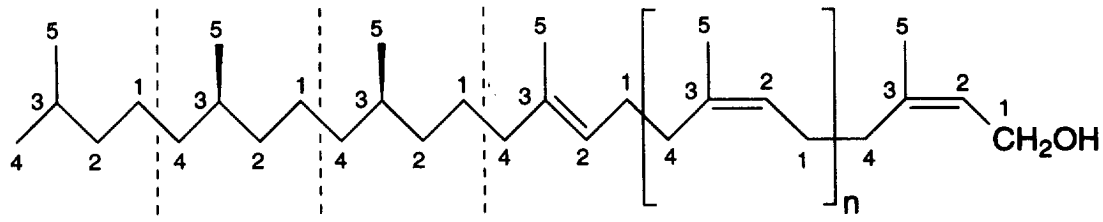
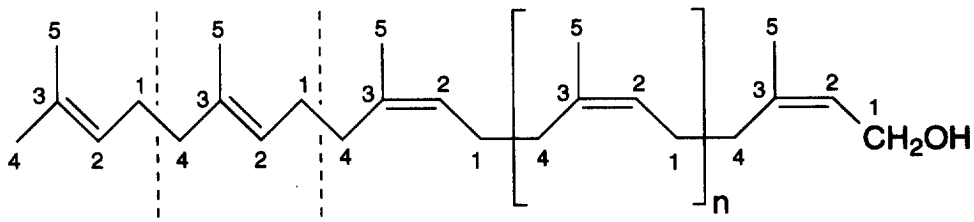


1 : n = 3
3 : n = 4
6 : n = 5
9 : n = 6
12 : n = 7



ω ψ χ *E* *Z* α(*Z*)

2 : n = 2
4 : n = 3
7 : n = 4
10 : n = 5



ω *E* χ(*Z*) *Z* α(*Z*)

5 : n = 6
8 : n = 7
11 : n = 8

of seven internal (*Z*)-prenyl residues [5*Z* and 5χ(*Z*)] and one ω-end prenyl residue (4ω). The methyl proton signal at δ 1.74 (3H, *br s*) indicated the presence of an α-terminal residue having the *Z*-configuration [3]. The relative intensities amongst these methyl proton signals suggested that compound **5** possessed one ω-end prenyl residue, one internal (*E*)-prenyl residue, seven internal (*Z*)-prenyl residues and one *Z* α-terminal prenyl residue aligned in that order. This hypothesis was supported by the relative intensities of the ^{13}C signals obtained with a nuclear Overhauser enhancement suppressed ^{1}H decoupled spectrum [3]. The relative intensity among the ^{13}C signals at δ 31.93, 32.16 and 39.71, ascribed to methylene carbons at 4χ(*Z*), 4*Z* and 4α(*Z*) and 4*E* (Table 1), respectively, was determined to be 1:7:1. These findings clearly indicated that compound **5** is comprised of a geranyl residue, seven internal (*Z*)-prenyl residues and a *Z* α-terminal prenyl residue aligned in that order. Thus, the structure of arachisprenol-10 (**5**) was established to be (2*Z*,6*Z*,10*Z*,14*Z*,18*Z*,22*Z*,26*Z*,30*Z*,34*E*)-3,7,11,15,19,23,27,31,35,39-decamethyl-2,6,10,14,18,22,26,30,34,38-tetracontadecaen-1-ol.

Arachisprenols 11 (**8**) and 12 (**11**) showed a $[M]^+$ at *m/z* 766.7017 ($[C_{55}H_{90}O]^+$) and 834.7618 ($[C_{60}H_{98}O]^+$) in their high-resolution EI-mass spectra, respectively. These data indicated that **8** and **11** are C_{55} and c_{60} homologues of compound **5**, respectively. This was supported by the similarity of the ^{1}H NMR spectra of compounds **8** and **11** to that of compound **5**, except for the integral values of the signals at δ 1.68, 2.00–2.09 and 5.12, which are assigned to the methyl, meth-

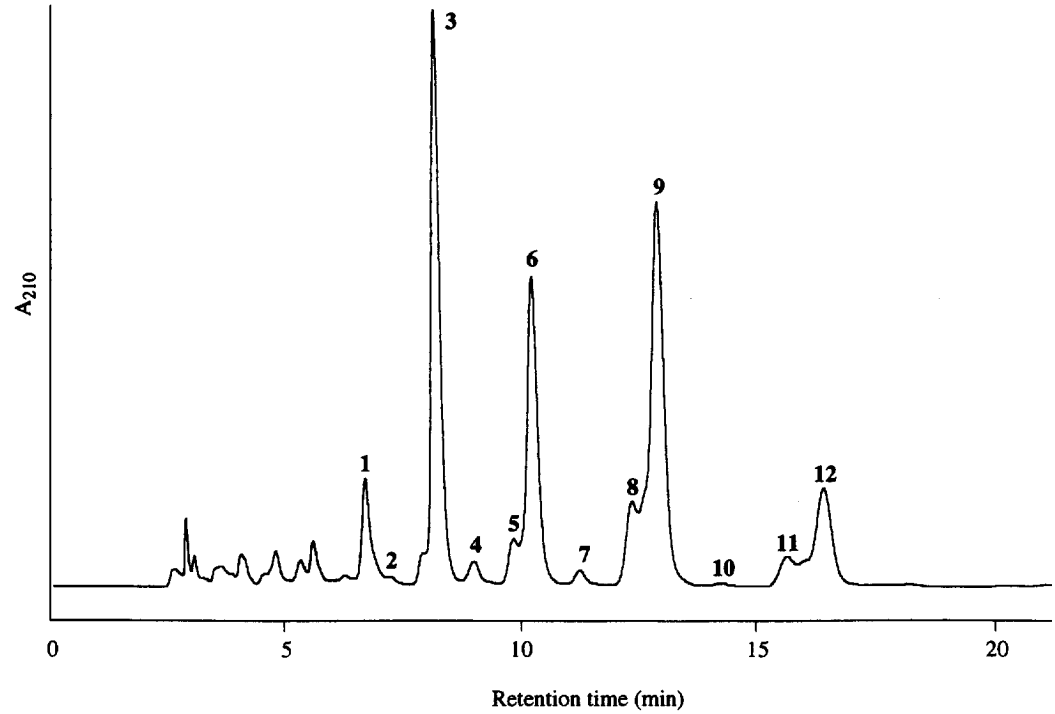


Fig. 1. Reverse-phase HPLC of polyprenol fraction from peanut leaves, eluted with hexane–methanol (1:4).

ylene and olefinic protons of the internal (*Z*)-prenyl residues, respectively. The difference between the integral values suggested that compounds **8** and **11** have eight and nine internal (*Z*)-prenyl residues, respectively. This suggestion was confirmed by comparison between the ^{13}C NMR spectra of compounds **8** and **11** with that of compound **5** (Table 1). These clearly indicated that arachisprenols 10 (**5**), 11 (**8**) and 12 (**11**) have the same geranyl and *Z* α-terminal residues and differ only in the number of internal (*Z*)-prenyl residues.

Table 1. ^{13}C NMR chemical shifts of arachisprenols **5**, **8** and **11**

Position	**5**	**8**	**11**
3α(*Z*)	139.8	139.9	139.9
3*E*,3*Z*,3χ(*Z*)	136.0	136.1	136.1
	135.3 × 2	135.4 × 2	135.4 × 2
	135.2 × 4	135.3	135.3 × 2
	135.1	135.2 × 4	135.2 × 6
		135.1	
3ω	131.3	131.3	131.3
2*Z*,2α(*Z*),2χ(*Z*)	125.0 × 3	125.0 × 4	125.0 × 5
	124.9 × 3	124.9 × 3	124.9 × 2
	124.5	124.5	124.5 × 3
	124.4	124.4	
2*E*	124.3	124.3	124.4
2ω	124.1	124.1	124.2
1α(*Z*)	59.0	59.0	59.0
4*E*	39.7	39.7	39.7
4*Z*,4α(*Z*)	32.2 × 7	32.2 × 8	32.2 × 9
4χ(*Z*)	31.9	31.9	31.9
1ω	26.7	26.7	26.7
1*E*	26.6	26.6	26.6
1*Z*,1χ(*Z*)	26.4 × 5	26.4 × 6	26.4 × 9
	26.3 × 2	26.3 × 2	
4ω	25.6	25.7	25.7
5*Z*,5α(*Z*),5χ(*Z*)	23.4 × 8	23.4 × 9	23.4 × 10
5ω	17.6	17.7	17.7
5*E*	15.9	16.0	15.9

Glycinoprenol 8 (**4**) gave a $[M]^+$ at *m/z* 568.5551 ($[C_{40}H_{72}O]^+$) in its high-resolution EI-mass spectrum. Comparison between the mass, ^1H NMR and ^{13}C NMR (Table 2) spectral data of compound **4** with those of glycinoprenols 9 (**7**) and 10 (**10**) [13] indicated that compound **4** was C_{40} glycinoprenol homologue comprised of a phytyl residue, an internal (*E*)-prenyl residue, three internal (*Z*)-prenyl residues and a *Z* α-terminal residue aligned in that order. Compound **4** has two chiral centres at C-23 and C-27 in the phytyl residue. Ozonolysis of compound **4** gave 6,10,14-trimethylpentadeca-2-one (**13**); the configurations of the two chiral centres at C-6 and C-10 originating from C-23 and C-27 of compound **4** were *R*, as deduced from a combination of CD [25], GC and ^{13}C NMR analyses, as established in the case of glycinoprenol 10 [13]. Because C-6 and C-10 of compound **13** originate from C-23 and C-27 of compound **4**, respectively, the configurations at C-23 and C-27 of compound **4** are concluded to be all *R*. Consequently, the structure of compound **4** is (23*R*,27*R*)-(2*Z*,6*Z*,10*Z*,14*Z*,18*E*)-3,7,11,15,19,23,27,31-octamethyl-2,6,10,14,18-dotriacontapentaen-1-ol.

Glycinoprenol 7 (**2**) gave a $[M]^+$ at *m/z* 500.5005 ($[C_{35}H_{64}O]^+$). Its mass spectral data indicated that it was a C_{35} homologue of glycinoprenol 8 (**4**), 9 (**7**)

Table 2. ^{13}C NMR chemical shifts of glycinoprenols **2** and **4**

Position	2	4
3α(*Z*)	139.6	139.9
3*E*,3*Z*	135.6	136.1
	135.4	135.6
	135.3	135.4 × 2
2*Z*,2α(*Z*)	124.4	124.9
	124.3	124.5
	124.2	124.4
		124.2
2*E*	123.9	123.9
1α(*Z*)	59.0	59.0
4*E*	40.1	40.0
2ω	39.4	39.4
2χ,2ψ,4χ,4ψ	37.5 × 2	37.4 × 2
	37.3	37.3
	36.7	36.7
3χ,3ψ	32.8 × 2	32.8
		32.7
4*Z*,4α(*Z*)	32.2 × 2	32.2 × 3
	32.0	32.0
3ω	28.0	28.0
1*E*	26.7	26.7
1*Z*	26.4 × 2	26.4 × 3
1χ,1ψ,1ω	25.4	25.4
	24.8	24.8
	24.5	24.5
5*Z*,5α(*Z*)	23.5 × 2	23.5 × 2
	23.4	23.4 × 2
4ω,5ω	22.7 × 2	22.7
		22.6
5χ,5ψ	19.8 × 2	19.8 × 2
5*E*	16.1	16.0

and 10 (**10**). This assumption was supported by the similarity of the ^1H NMR and ^{13}C NMR (Table 2) data of compound **2** and compounds **4**, **7** and **10**, except for the integral values of the proton and carbon signals due to an internal (*Z*)-prenyl residue. Thus, compound **2** comprised a phytyl residue, an internal (*E*)-prenyl residue, two internal (*Z*)-prenyl residues and a *Z* α-terminal residue aligned in that order.

Ficaprenols 8 (**1**), 9 (**3**), 10 (**6**), 11 (**9**) and 12 (**12**) and glycinoprenol 9 (**7**) and 10 (**10**) were identified by comparison of their retention times with those of authentic samples on reverse-phase HPLC. Identity was also established by direct comparison of their IR, ^1H NMR, ^{13}C NMR and mass spectra with those of known compounds.

The contents of arachisprenols, glycinoprenols and ficaprenols in the leaves of peanuts increased gradually during 16 weeks after germination; their ultimate contents reached 0.045, 0.025 and 0.23% of the fresh leaves, respectively, just before the leaves turned yellow.

Thus far, polyprenols having a geranylgeranyl residue, a farnesyl residue, a phytyl residue or two saturated prenyl residues at the ω-end of their prenyl chain have been isolated. These polyprenols are all (*E*)- and (*Z*)-mixed type polyprenols having two or three internal (*E*)-prenyl residues. The three novel polyprenols, arachisprenols 10–12 isolated from peanut leaves comprised a geranyl residue, seven to nine (*Z*)-prenyl residues and a *Z* α-terminal residue aligned in that order. The occurrence of C_{50}-, C_{55}- and C_{60}-polyprenols having a geranyl residue has been demonstrated for the first time.

EXPERIMENTAL

General. ^1H NMR and ^{13}C NMR spectra were obtained with JEOL JNM GSX 270 and JNM GSX-500 spectrometers at 270.2 and 500.2 MHz for ^1H NMR and 67.9 and 125.8 MHz for ^{13}C NMR. Measurements were made in $CDCl_3$ at 27° and the 7.26 ppm resonance of residual $CHCl_3$ and 77.0 ppm of $CDCl_3$ were used as int. refs for ^1H NMR and ^{13}C NMR, respectively. Relative intensities of carbon signals were obtained using nuclear Overhauser enhancement-suppressed ^1H decoupling and multiple scans at a pulse repetition time of 15 sec. MS were obtained at 70 eV. GC-MS were recorded on the same spectrometer using an OV-1 fused capillary column (0.2 mm × 50 m); column temp. 100–230° at 0.5° min^{-1}. Analytical GC was carried out with FID under the same conditions.

Isolation and purification of polyprenols. Leaves (1.2 kg) of peanuts (*A. hypogaea* L.) were collected at the end of September (20 weeks after germination) and immersed in MeOH (3 l) at room temp. for 2 weeks. The MeOH soln was concd to 1.3 l and dild with H_2O (300 ml), followed by extraction with hexane. Removal of solvent gave a brown viscous oil (10.2 g), which was subjected to silica gel CC with benzene as eluent to afford a polyprenol fr. (1.83 g). This was subjected to HPLC analysis on a Wakosil 5C18 column (4.6 mm i.d. × 250 mm), showing 12 peaks (Fig. 1). Separation of each component was carried out by means of the reverse-phase HPLC on a Zorbax-ODS column (21.2 mm i.d. × 250 mm) with hexane–MeOH (1:4). Each component obtained was further purified by repeated reverse-phase HPLC on a Wakosil 5C18 column (4.6 mm i.d. × 250 mm) with hexane–MeOH (1:4) to give 5, 1, 145, 6, 9, 58 3, 8, 83, 1, 2 and 12 mg of pure compounds **1**–**12**, respectively. All isolated compounds showed a single peak by HPLC (ODS; hexane–MeOH, 1:4). The content of the polyprenols varied with growth of the plant: age of leaves expressed in week after germination (wt% of arachispenols in the fr. leaves, wt% of glycinoprenols in fr. leaves and wt% of ficaprenols in fr. leaves): 2 (0, 0, 0), 4 (0, 0, 0.001), 8 (0.001, 0.001, 0.02), 10 (0.002, 0.002, 0.04), 12 (0.02, 0.004, 0.11), 14 (0.04, 0.02, 0.19), 16 (0.045, 0.025, 0.23) and 18 (0.01, 0.01, 0.07).

Identification of ficaprenols 8 (**1**), 9 (**3**), 10 (**6**), 11 (**9**) *and* 12 (**12**) *and glycinoprenols* 9 (**7**) *and* 10 (**10**). Compounds **1**, **3**, **6**, **9** and **12** and compounds **7** and **10** showed the same R_fs as authentic samples of ficaprenols 8–12 and glycinoprenols 9 and 10, respec-

tively, on reverse-phase HPLC (Wakosil 5C18 column). Identity was established by direct comparison of their IR, ^{1}H NMR, ^{13}C NMR and high-resolution mass spectra with those of authentic specimens.

Arachisprenol 10 (**5**). Colourless oil. IR ν_{max}^{film} cm^{-1}: 3330 (OH), 1665 (C=C). ^{1}H NMR: δ 1.60 (6H, *br s*, Me × 2), 1.68 (24H, *br s*, Me × 8), 1.74 (3H, *br s*, Me), 2.00–2.09 (37H, *m*, —CH_2 × 18 and OH), 4.10 (2H, *br*), 5.12 (9H, *m*), 5.44 (1H, *br t*, J = 7.3 Hz). ^{13}C NMR: Table 1. EI MS *m/z* (rel. int.): 698 (1, $[M]^+$), and 680 (2, $[M-H_2O]^+$), 121 (27), 109 (22), 107 (22), 95 (30), 93 (28), 81 (62), 69 (100); HR MS *m/z*: 698.6304 ($C_{50}H_{82}O$ requires *m/z* 698.6366), 680.6254 ($C_{50}H_{80}$ requires *m/z* 680.6260).

Arachisprenol 11 (**8**). Colourless oil. IR ν_{max}^{film} cm^{-1}: 3330 (OH), 1665 (C=C). ^{1}H NMR: δ 1.60 (6H, *br s*, Me × 2), 1.68 (27H, *br s*, Me × 9), 1.74 (3H, *br s*, Me), 2.00–2.09 (41H, *m*, —CH_2 × 20 and OH), 4.09 (2H, *br d*, J = 7.3 Hz), 5.12 (9H, *m*), 5.45 (1H, *br t*, J = 7.3 Hz). ^{13}C NMR: Table 1. EI MS *m/z* (rel. int.): 766 (1, $[M]^+$), 748 (3, $[M-H_2O]^+$), 121 (30), 109 (25), 107 (22), 95 (31), 93 (28), 81 (67), 69 (100); HR MS *m/z*: 766.7017 ($C_{55}H_{90}O$ requires *m/z* 766.6992), 748.6893 ($C_{55}H_{88}$ requires *m/z* 748.6886).

Arachisprenol 12 (**11**). Colourless oil. IR ν_{max}^{film} cm^{-1}: 3330 (OH), 1665 (C=C). ^{1}H NMR: δ 1.61 (6H, *br s*, Me × 2), 1.68 (30H, *br s*, Me × 10), 1.74 (3H, *br s*, Me), 2.00–2.09 (45H, *m*, —CH_2 × 22 and OH), 4.09 (2H, *br*), 5.12 (9H, *m*), 5.45 (1H, *br t*, J = 7.3 Hz). ^{13}C NMR: Table 1. EI MS *m/z* (rel. int.): 834 (1, $[M]^+$), 816 (2, $[M-H_2O]^+$), 121 (28), 109 (23), 107 (19), 95 (30), 93 (27), 81 (68), 69 (100); HR MS *m/z*: 834.7618 ($C_{60}H_{98}O$ requires *m/z* 834.7618), 816.7480 ($C_{60}H_{96}$ requires *m/z* 816.7512).

Glycinoprenol 7 (**2**). Colourless oil. IR ν_{max}^{film} cm^{-1}: 3330 (OH), 1665 (C=C). ^{1}H NMR: δ 0.84 (6H, *d*, J = 6.4 Hz, Me × 2), 0.86 (6H, *d*, J = 6.4 Hz, Me × 2), 1.00–1.52 (19H, *m*, —CH_2 and —CH), 1.60 (3H, *br s*, Me), 1.68 (6H, *br s*, Me × 2), 1.75 (3H, *br s*, Me), 2.00–2.09 (14H, *m*, —CH_2 × 7), 4.08 (2H, *br*), 5.11 (3H, *m* > C=CH), 5.44 (1H, *br t*, J = 7.3 Hz). ^{13}C NMR: Table 2. EI MS *m/z* (rel. int.): 500 (3, $[M]^+$), 482 (7, $[M-H_2O]^+$), 121 (35), 109 (42), 95 (65), 81 (100), 57 (98); HR MS *m/z*: 500.5005 ($C_{35}H_{64}O$ requires *m/z* 500.4957), 482.4795 ($C_{35}H_{62}$ requires *m/z* 482.4852).

Glycinoprenol 8 (**4**). Colourless oil. IR ν_{max}^{film}: 3330 (OH) 1665 (C=C). ^{1}H NMR: δ 0.84 (6H, *d*, J = 6.4 Hz, Me × 2), 0.86 (6H, *d*, J = 6.4 Hz, Me × 2), 1.00–1.52 (19H, *m*, —CH_2 and —CH), 1.60 (3H, *br s*, Me), 1.68 (9H, *br s*, Me × 3), 1.74 (3H, *br s*, Me), 2.00–2.09 (18H, *m*, —CH_2 × 9), 4.08 (2H, *br*), 5.11 (4H, *m* > C=CH), 5.44 (1H, *br*, *t*, J = 7.3 Hz). ^{13}C NMR: Table 2. EI MS *m/z* (rel. int.): 568 (2, $[M]^+$), 550 (7, $[M-H_2O]^+$), 121 (32), 109 (35), 95 (48), 81 (80), 57 (100); HR MS *m/z*: 568.5551 ($C_{40}H_{72}O$ requires *m/z* 568.5583), 550.5538 ($C_{40}H_{70}$ requires *m/z* 550.5478).

Ozonolysis of glycinoprenol 8 (**4**). According to the procedure used for ozonolysis of glycinoprenol 10 [13], ozonolysis of compound **4** was carried out to give compound **13**. Colourless oil. ν_{max}^{film} cm^{-1}: 1720 (C=O). ^{1}H NMR: δ 0.84 (6H, *d*, J = 6.5 Hz, Me × 2), 0.87 (6H, *d*, J = 6.5 Hz, Me × 2), 1.00–1.80 (19H, *m*, —CH_2 and —CH), 2.15 (3H, *s*, Me), 2.18 (1H, *br t*, J = 6.7 Hz, —CH_2CO—); ^{13}C NMR: δ 209.49 (C-2), 44.17 (C-3), 39.37 (C-13), 37.41 (C-11), 37.29 (C-9), 37.23 (C-7), 36.49 (C-5), 32.80 (C-10), 32.68 (C-6), 29.72 (C-1), 27.99 (C-14), 24.79 (C-12), 24.42 (C-8), 22.73 (C-18), 22.63 (C-15), 21.42 (C-4), 19.76 (C-17), 19.58 (C-16). EI MS *m/z* (rel. int.): 268 (1, $[M]^+$), 253 (1, $[M-Me]^+$) and 43 (100); CD $\Delta\varepsilon_{284}$ + 0.07 ± 0.02 (hexane; *c* 0.01). Compound **13** was identified as (6*R*,10*R*)-6,10,14-trimethylpentadecan-2-one by direct comparison of CD, IR, ^{13}C NMR, ^{1}H NMR and EI-MS spectra with those of an authentic sample and a racemic mixt. [(6*R*,10*R*)-, (6*S*,10*S*)-, (6*R*,10*S*)- and (6*S*,10*R*)-**13**], together with GC co-injection analysis, with compounds prepared from ozonolysis of natural (7*R*,11*R*)-phytol and hydrogenation of 6,10,14-trimethyl-5,9,13-pentadecatrien-2-one, respectively [13].

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