Pergamon

PII: S0031-9422(96)00268-3

Phytochemistry, Vol. 43, No. 1, pp. 179–182, 1996

0031-9422/96 $15.00 + 0.00

TWO PENTACYCLIC TRITERPENES FROM *PHYTOLACCA DODECANDRA* ROOTS

SIGRID M. SPENGEL

School of Pharmacy of the University of Basel, Totengässlein 3, 4051 Basel, Switzerland

(*Received in revised form* 26 *March* 1996)

Key Word Index—*Phytolacca dodecandra*; Phytolaccaceae; triterpenoids; olean-12-ene; dodecandral; dodecandralol.

Abstract—From the dried roots of *Phytolacca dodecandra*, the three known olean-12-ene-dicarboxylic acids phytolaccagenin, phytolaccagenic acid and serjanic acid and two new genins, named as dodecandral and dodecandralol, have been isolated and characterized. The two new compounds have at C-20 an aldehyde function instead of a carboxyl group or its methyl ester. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The interest in *Phytolacca dodecandra* l'Hérit = Endod is based on the high molluscicidal activity of its unripe berries. There are great expectations in the potential control of the tropical parasitic disease schistosomiasis by interrupting the life cycle of the parasites by elimination of aquatic snails [1] using a safe, non-toxic and inexpensive, plant derived molluscicide. The snails (e.g. *Biomphalaria glabrata*) serve as intermediate hosts of schistosomal cercariae.

So far compounds **1–4** are the only genins isolated from *P. dodecandra* [2]. They were found in different percentages, varying from *ca* 45 to 95% for the main constituent oleanolic acid (**1**), depending on the various plant types from several geographical regions of Africa [3]. Recently, we showed the presence of 28,30-dicarboxy-olean-12-enes in *P. dodecandra* leaves, roots, berries and calli by HPLC analysis [4]. Phytolaccagenin and phytolaccagenic acid and their glycosides, esculentosides B, S and L_1, have been isolated from calli initiated from leaves of *P. dodecandra* [5, 6]. From the HPLC analysis, we knew that the 28-mono-carboxy-olean-12-enes oleanolic acid (**1**), hederagenin (**3**) and bayogenin (**4**) are present in roots besides the 28,30-dicarboxy-olean-12-enes phytolaccagenic acid (**6**) and phytolaccagenin (**7**). In the present investigation, we were interested only in the isolation of 28,30-dicarboxy-olean-12-enes. In addition to the three dicarboxylic acids **5–7** we also isolated and identified the two new substances, **8** and **9**, with an aldehyde group at C-20 instead of a carboxylic acid or its methyl ester.

RESULTS AND DISCUSSION

The dried and ground roots were extracted with methanol. The extract was partitioned between water and *n*-butanol. After distilling off the alcohol the residue was hydrolysed in 2 N HCl. The precipitated genins were separated by filtration and subjected to flash chromatography.

In addition to the known compounds **5–7**, we found that two new substances, **8** and **9**, had been eluted together with serjanic acid (**5**) and **6**, respectively. The separation of **5** and **8** as well as **6** and **9** was carried out on RP 18 flash silica gel. The ^{1}H NMR spectrum of **8**, like that of **5**, exhibited signals due to six tertiary methyl groups at δ 0.90–1.31, whereas the spectrum of **9**, like **6**, displayed only five tertiary methyl groups between δ 0.93 and 1.26. This led to the hypothesis that there could be a close relationship between the co-eluted pairs of substances. In the ^{1}H NMR spectrum both compounds revealed four isolated protons due to the typical signals of H-18 (1H, *dd*) [7], H-3 (1H, *t*), H-12 (1H, '*t*') and at δ 9.6 a signal (1H, *s*) corresponding to an aldehyde. The triplet for the H-3 in **8** appeared at δ 3.48 and in **9** at δ 4.24. The C-23 α-oriented hydroxymethylene, represented by an AB coupling (J = 10.3 Hz) with one doublet centred at δ 3.74 and the other at δ 4.22, was responsible for the downfield shift of H-3 as it could be observed in **3**, **4**, **6** and **7**. The chemical shift of the signal for the olefinic proton at C-12 was similar in both compounds, i.e. in **8** at δ 5.56 and in **9** at δ 5.57. From the ^{13}C NMR spectra of **8** and **9** (Table 1) we knew that in addition to the aldehyde signal at δ 206.4 there was a carboxyl signal at δ 180.3 or 179.5, respectively.

H-18 could be used for further structural elucidation of **8** and **9**. Thus, if there was a carboxyl group at C-17, the H-18 signal would be a doublet of a doublet with J_1 = 4.1–4.3 Hz and J_2 = 13–14 Hz. Using $CDCl_3$ as solvent for recording the ^{1}H NMR spectrum, the signal was centred at δ 2.85 for the free acid as well as for the ester. With pyridine-d_5 as solvent, the double doublet

Table 1. ^{13}C NMR spectral data for compounds **8** and **9** (50.3 MHz, δ in pyridine-d_5)

C	8	9
1	39.0	38.9
2	28.2	27.7
3	78.1	73.5
4	39.4	42.9
5	55.6	48.7
6	18.8	18.6
7	33.3	33.0
8	39.8	39.8
9	48.2	48.2
10	37.4	37.3
11	23.7	23.9
12	122.4	123.8
13	144.6	144.3
14	42.2	42.2
15	28.4	28.4
16	23.8	23.6
17	46.3*	46.2*
18	43.2	43.1
19	40.4	40.2
20	46.9*	46.9*
21	28.2	28.0
22	33.9	33.8
23	28.8	68.1
24	16.6	13.1
25	15.6	16.0
26	17.5	17.5
27	26.4	26.4
28	180.3	179.5
29	24.2	24.1
30	206.4	206.3

*Assignments may be reversed.

was centred at δ 3.32–3.35. This was true for **1–7** and must be due to a reaction of pyridine with the acidic group. When the 28-COOH group was methylated, the signal for H-18 migrated upfield by 0.2 ppm. A reaction of pyridine with the ester was no longer possible. When the dimethyl ester of **7** was prepared (H-18 of **7**: δ 3.34; H-18 of the dimethyl ester of **7**: δ 3.14) and partially saponified (C-30) with KOH [8], we obtained 28-methyl-jaligonate. This compound had the H-18 (in pyridine-d_5) centred at δ 3.45. If remethylated to the dimethyl ester the signal of H-18 migrated to δ 3.13. In glycosides with the sugar bound to the C-3 hydroxyl group, we had the same difference of 0.2 ppm in the chemical shift of H-18 between the free acid at C-17 and its ester. If the 28-COOH had an ester glycosidic linked sugar, again (in pyridine-d_5) H-18 had an upfield shift, in this case of *ca* 0.1 ppm. The ^1H NMR spectrum of **8** recorded in $CDCl_3$ showed the H-18 signal centred at δ 2.63 and its methyl ester at δ 2.66, but if it was recorded in pyridine-d_5 the H-18 signal appeared at δ 3.24 and its methyl ester at δ 3.02 (cf. H-18 of **9** at δ 3.20). Because of this shift of 0.2 ppm, as described above, the carboxyl group was attributed to C-17.

Ikuta and Itokawa [9] isolated 3β-hydroxy-29(or 30)-al-olean-12-ene-28-oic acid from callus cultures of *Akebia quinata* and 3β-hydroxy-29-al-olean-12-ene-28-oic acid [10] from callus cultures of *Stanntonia hexaphylla*. They considered the aldehyde to be an intermediate in the oxidation of oleanolic acid to mesembryanthemoidigenic acid (29-CH_2OH) and finally seragenic acid (29-COOH). It has been demonstrated that if the equatorial (α, C-29) methyl group at C-20 has an oxygenated function, the axial (β, C-30) methyl is deshielded and appears at *ca* δ 19 [10–13]. In the ^{13}C NMR spectra of **8** and **9** there was no signal for a methyl group at δ 19, but a shift of δ 24.1 and, therefore, most likely there was no equatorial, but an axial aldehyde attached to C-20 of the triterpene skeleton. The signals at δ 46.3 and 46.9 for two quaternary carbon atoms were assigned to C-17 and C-20, respectively, indicating that in both cases oxygenated methyl groups were attached. Since we knew from the shift of H-18 in the ^1H NMR spectrum that the carboxyl group must be located at C-17 the aldehyde group must be on C-20.

For both substances **8** and **9**, the EI-mass spectrum produced the same fragmentation pattern as expected for olean-12-enes. At *m/z* 262 we had the retro-Diels–Alder (RDA) fragment, indicating that in both compounds the carboxyl and the aldehyde group are attached to rings D and E. The loss of an aldehyde group from the RDA fragment gave *m/z* 233, the loss of 46 amu (COOH + H) from *m/z* 262 led to *m/z* 216 and then to *m/z* 187 $[216 - CHO]^+$. In the case of **8** *m/z* 207 was the fragment of ring A and B and in the case of **9** it was at *m/z* 223, indicating that in **9** this fragment was carrying two hydroxyl groups.

From all these results, the structure of **8** was confirmed as 3β-hydroxy-30-al-olean-12-ene-28-oic acid and of **9** as 3β,23-dihydroxy-30-al-olean-12-ene-28-oic acid. Both substances are described for the first time and are named as dodecandral and dodecandralol, respectively.

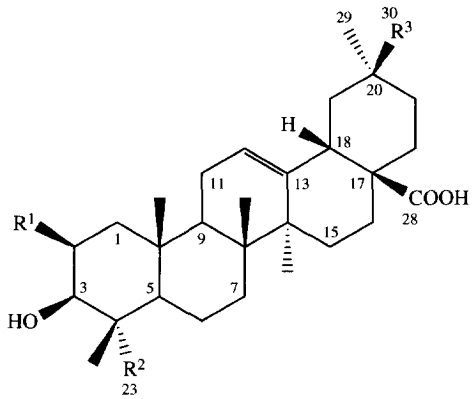
EXPERIMENTAL

NMR: 200 MHz (^1H) and 50.3 MHz (^{13}C) in pyridine-d_5 with TMS as int. standard; MS: EI 70 eV; TLC: silica gel GUV_{254}, 0.5 mm thickness for prep. TLC; CC: silica gel and RP 18 silica gel for flash CC; mps: uncorr.

Plant material. Phytolacca dodecandra was grown in a greenhouse in the Botanical Garden in Brueglingen near Basel [5].

Extraction. Dried and ground (15.0 g) roots were extracted (×2) at 35–40° for 5 hr with 150 ml MeOH each time. The solvent was removed *in vacuo* yielding 4.64 g of extract. This residue was suspended in H_2O and the saponins were exhaustively extracted with *n*-BuOH satd with H_2O.

Hydrolysis and isolation. The saponin mixt. (2.07 g) was hydrolysed in 30 ml 2 N HCl at 100° for 3 hr. The



	R^1	R^2	R^3	Trivial name
1	H	Me	Me	Oleanolic acid
2	OH	Me	Me	2-Hydroxy-oleanolic acid
3	H	CH_2OH	Me	Hederagenin
4	OH	CH_2OH	Me	Bayogenin
5	H	Me	COOMe	Serjanic acid
6	H	CH_2OH	COOMe	Phytolaccagenic acid
7	OH	CH_2OH	COOMe	Phytolaccagenin
8	H	Me	CHO	Dodecandral
9	H	CH_2OH	CHO	Dodecandralol

mixt. was adjusted to pH 3 and the ppt. (1.28 g) was isolated from the aq. soln by filtration and subjected to flash CC on silica gel, eluting with $CHCl_3$–MeOH mixts. The elution yielded **1** (104 mg), **5** and **8** (32 mg), **3**, **6** and **9** (172 mg) and **7** (275 mg). The crude mixt. of **5** and **8** was repeatedly subjected to silica gel prep. TLC [$CHCl_3$–MeOH (19:1, 97:3) or petrol–EtOAc (7:3), respectively], and finally on RP 18 with MeOH–H_2O (17:3) as eluent, giving **5** (12 mg) and **8** (2 mg). The mixt. of **3**, **6** and **9** was further purified by flash CC with a 1:1 mixt. of $CHCl_3$–MeOH (19:1) and Et_2O and by prep. TLC with the same solvent, yielding **3** (37 mg) and **3**, **6** and **9** (73 mg). Further sepn was done by flash CC on RP 18 with H_2O–MeCN–MeOH (50:50:3), giving **6** (14 mg), and again with the same solvent (4:15:1), yielding crude **9** (10 mg) and a mixt. of **6**, **9** and **3** (40 mg).

Serjanic acid (**5**). Crude **5** (12 mg) gave 8 mg crystals from $CHCl_3$–petrol, mp 279–282°. ^{1}H NMR and MS were compared with those form the lit. [14] and shown to be identical. The methyl ester data were also identical with published data [15].

Phytolaccagenic acid (**6**). 10 mg from MeOH–Et_2O–petrol from 14 mg crude **6**, mp 280–284°. The comparison with authentic material showed identity.

Phytolaccagenin (**7**). Compound **7** (275 mg) were crystallized from *i*-PrOH-petrol: 198 mg, mp 280–290° and recrystallized from MeOH: 143 mg, mp 283–287°. Mp, TLC, MS, ^{1}H and ^{13}C NMR were identical with those of authentic material.

Dodecandral (**8**). Non-crystalline. ^{1}H NMR: δ 0.90 (3H, *s*), 0.96 (3H, *s*), 1.01 (3H, *s*), 1.06 (3H, *s*), 1.28 (3H, *s*), 1.31 (3H, *s*), 3.24 (1H, *dd*, $J_1 = 4.3$ Hz, $J_2 =$ 13.7 Hz, H-18), 3.48 (1H, *t*, $J = 7.8$ Hz, H-3), 5.56 (1H, '*t*', H-12), 9.67 (1H, *s*, CHO). Molecular formula: $C_{30}H_{46}O_4$ 470. EIMS 70 eV, *m/z* (rel. int.): 470 (0.7) $[M]^+$, 424 (4.3) $[M - CO_2H - H]^+$, 262 (100) RDA fragment of rings D and E, 234 (30.6), 233 (29.4) $[262 - CHO]^+$, 216 (72.7) $[262 - CO_2H - H]^+$, 207 (64.7) RDA fragment of rings A and B, 189 (44.7) $[207 - H_2O]^+$, 187 (56.5) $[262 - HCO_2H - CHO]^+$.

Dodecandral methyl ester. Compound **8** (1 mg) in 0.1 ml MeOH was methylated with freshly prepd CH_2N_2. ^{1}H NMR: δ 0.93 (3H, *s*), 0.95 (3H, *s*), 1.07 (3H, *s*), 1.24 (3H, *s*), 1.26 (6H, *s*), 3.02 (1H, *dd*, $J_1 = 4.25$ Hz, $J_2 = 10.1$ Hz, H-18), 3.47 (1H, *t*, $J =$ 7.3 Hz, H-3), 3.66 (3H, *s*, OMe), 5.48 (1H, '*t*', H-12), 9.61 (1H, *s*, CHO).

Dodecandralol (**9**). Non-crystalline. ^{1}H NMR: δ 0.93 (3H, *s*), 0.97 (3H, *s*), 1.03 (3H, *s*), 1.07 (3H, *s*), 1.26 (3H, *s*), 3.20 (1 H, *dd*, $J_1 = 4.1$ Hz, $J_2 = 13.3$ Hz, H-18), 3.74 (1H, *d*, $J = 10.3$ Hz), 4.22 (1H, *d*, $J =$ 10.3 Hz, H_2-23), 4.24 (1H, *t*, $J = 7.2$ Hz, H-3), 5.57 (1H, '*t*', H-12), 9.65 (1H, s, CHO). Molecular formula:

$C_{30}H_{46}O_5$ 486. EIMS 70 eV, *m/z* (rel. int.): 440 (3.0) $[M - COOH - H]^+$, 262 (74.6) RDA fragment of rings D and E, 234 (64.0), 233 (39.0) $[262 - CHO]^+$, 223 (18.1) RDA fragment of rings A and B 216 (48.9) $[262 - COOH - H]^+$, 205 (33.4) $[223 - H_2O]^+$, 187 (78.9) $[205 - H_2O]^+$ and/or $[262 - HCO_2H - CHO]^+$.

Acknowledgements—I am grateful to Prof. W. Schaffner for ceding me the plant material and to Prof. H. H. A. Linde for his helpful discussions of the spectra, both from the School of Pharmacy of the University of Basel, and to Dr H. Nadig, Institute for Organic Chemistry of the University of Basel, for recording the MS.

REFERENCES

1. Lambert, J. D. H., Temmink, J. H. M., Marquis, J., Parkhurst, R. M., Lugt, C. B., Lemmich, E., Wolde-Yohannes, L. and de Savigny, D. (1991) *Regul. Toxicol. Pharmacol.* **14**, 189.
2. Parkhurst, R. M. (1975) *Indian J. Chem.* **13**, 757.
3. Parkhurst, R. M., Thomas, D. W., Adams, R. P., Makhubu, L. P., Mthupa, B. M., Wolde-Yohannes, L., Mamo, E., Heath, G. E., Stobaeus, J. K. and Jones, W. O. (1990) *Phytochemistry* **29**, 1171.
4. Spengel, S. M., Luterbacher, S. and Schaffner, W. (1992) *Planta Med.* **58**, A 684.
5. Spengel, S. M., Luterbacher, S. and Schaffner, W. (1995) *Planta Med.* **61**, 385.
6. Luterbacher, S. (1995) Ph.D. Thesis. University of Basel, Switzerland.
7. Cheung, H. T. and Yan, T. C. (1972) *Austr. J. Chem.* **25**, 2003.
8. Woo, W. S., (1973) *Lloydia* **36**, 326.
9. Ikuta, A. and Itokawa, H. (1986) *Phytochemistry* **25**, 1625.
10. Ikuta, A. (1987) *J. Nat. Prod.* **52**, 623.
11. Coxon, D. T. and Wells, J. W. (1980) *Phytochemistry* **19**, 1247.
12. Harkar, S., Razdan, T. K. and Waight, E. S. (1984) *Phytochemistry* **23**, 2893.
13. Rogers, C. B. (1989) *J. Nat. Prod.* **52**, 528.
14. Razdan, T. K., Harkar, S., Kachroo, V., Koul, G. L. and Waight, E. S. (1983) *Phytochemistry* **22**, 1797.
15. Bandara, B. M. R., Jayasinghe, U. L. B., Karunaratne, V., Wannigama, G. P., Bokel, M., Kraus, W. and Sotheeswaran, S. (1990) *Planta Med.* **56**, 290.