Phytochemistry, Vol. 24, No. 12, pp. 3003–3006, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

A TRITERPENOID GLYCOSIDE FROM *TETRAPLEURA TETRAPTERA* FRUIT

SIMEON K. ADESINA* and JOHANNES REISCH

Institute of Pharmaceutical Chemistry, Westfalian Wilhelms-University, Hittorfstr. 58–62, 4400 Münster, West Germany

(*Revised received* 29 *March* 1985)

Key Word Index—*Tetrapleura tetraptera*; Mimosaceae; fruit; adridanin; 3-*O*-[β-D-glucopyranosyl-2′-acetamido-2′-deoxy]-oleanolic acid.

Abstract—The fruit pulp of *Tetrapleura tetraptera* has yielded aridanin, a novel 3-*O*-[β-D-glucopyranosyl-2′-acetamido-2′-deoxy]-oleanolic acid. Hentriacontane, phenylpropanoids and carbohydrate residues were also isolated and identified.

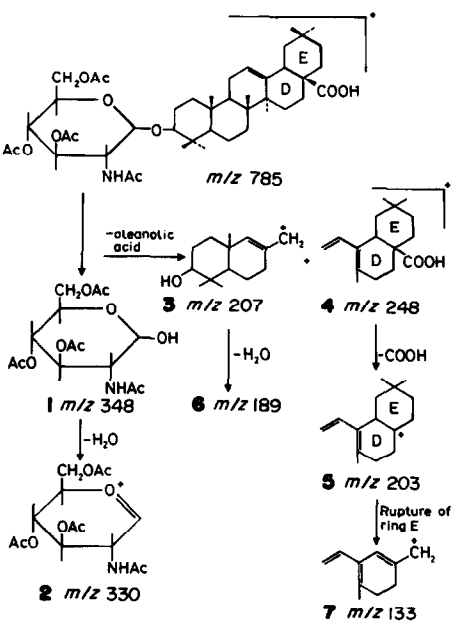
INTRODUCTION

The dark-brown fruits of *T. tetraptera* Taub., a well-known Nigerian drug (known locally as Aridan), have four longitudinal ridges of which two are hard and woody. The other two soft ridges are eaten in soup as a flavouring or in pot-herb as an anticonvulsant [Elewude, J. A., personal communication]. Recently, this drug was found to be a potent molluscicide [1] and this has stimulated research into its detailed chemistry. The fruits and stem bark were reported [2, 3] to contain tannin, traces of saponin and amino acids. The identification of scopoletin and its biological effects in experimental animals were reported recently [4, 5]. This paper deals with the isolation and structure elucidation of a new mono-*N*-acetyl glycoside for which the name aridanin is proposed. Aridanin is the major glycoside of Aridan fruit.

RESULTS AND DISCUSSION

Aridanin, white powder, mp 276–280° (dec.), $[\alpha]_D^{25}$ +40.96° (*c* 0.5; pyridine), afforded oleanolic acid and acetyl glucose on acid hydrolysis with 2% H_2SO_4–EtOH during 6 hr. Treatment of aridanin with $Ba(OH)_2$ [6] followed by acid hydrolysis of the deacetylated product led to the identification of oleanolic acid and glucose in a very poor yield.

Simple conventional methods of cleavage of the acetyl group failed suggesting a C–C or C–N linkage between the acetyl group and the carbohydrate moiety. Aridanin showed ^{1}H NMR signals ($CDCl_3 + CD_3OD$) at δ0.76, 0.78, 0.91, 0.93, 0.95, 1.13, 1.27 (Me × 7), 1.98 (acetyl, NAc), 4.47 (1H, *d*, *J* = 8.2 Hz, anomeric proton), 5.30 (1H, *m*, olefinic). It had an $[M]^+$ (FDMS) at *m/z* (rel. int.) 659 (8) and an $[M+1]^+$ at *m/z* 660 (7) for $C_{38}H_{61}NO_8$. Its peracetylated derivative showed ^{1}H NMR signals ($CDCl_3$) at δ1.92 (NAc), 2.01, 2.04, 2.06 (3 × OAc) (cf. ^{1}H NMR signals in pyridine-d_5, Fig. 1) and an $[M]^+$ (FDMS) at *m/z* (rel. int.) 785 (18) with $[M+1]^+$ at *m/z* 786 (100) for $C_{44}H_{67}NO_{11}$. An N–H signal (δ 8.9, *d*, $J_{2',NH}$ = 9.2 Hz, Fig. 1) appeared for both aridanin and its peracetylated derivative as expected. The CIMS of the per-acetylated derivative of aridanin gave fragments of *m/z* (rel. int.) 348 (50), 330 (85), 270 (11), 210 (56) and 150 (88) which are due to the carbohydrate residue. The ion at *m/z* 348 is due to the formation of 2-acetamido-1-hydroxy-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranose (**1**) (see Scheme 1) which, by the loss of H_2O, yields (**2**), the main ion at *m/z* 330. Per-acetylated carbohydrates normally furnish fragments corresponding to losses of one or more molecules of acetic acid and one of ketene per molecule [7]. Thus the rest of the spectrum can be rationalised by a loss of one (*m/z* 270), two (*m/z* 210) and three (*m/z* 150) molecules of acetic acid. The particular stability of the glycosyl cation (**2**) appears to be the factor determining the mode of fragmentation. As would be expected fragments were observed at *m/z* 228 (10%) and



Scheme 1.

*Permanent address: Drug Research & Production Unit, Faculty of Pharmacy, University of Ife, Ile-Ife, Nigeria.

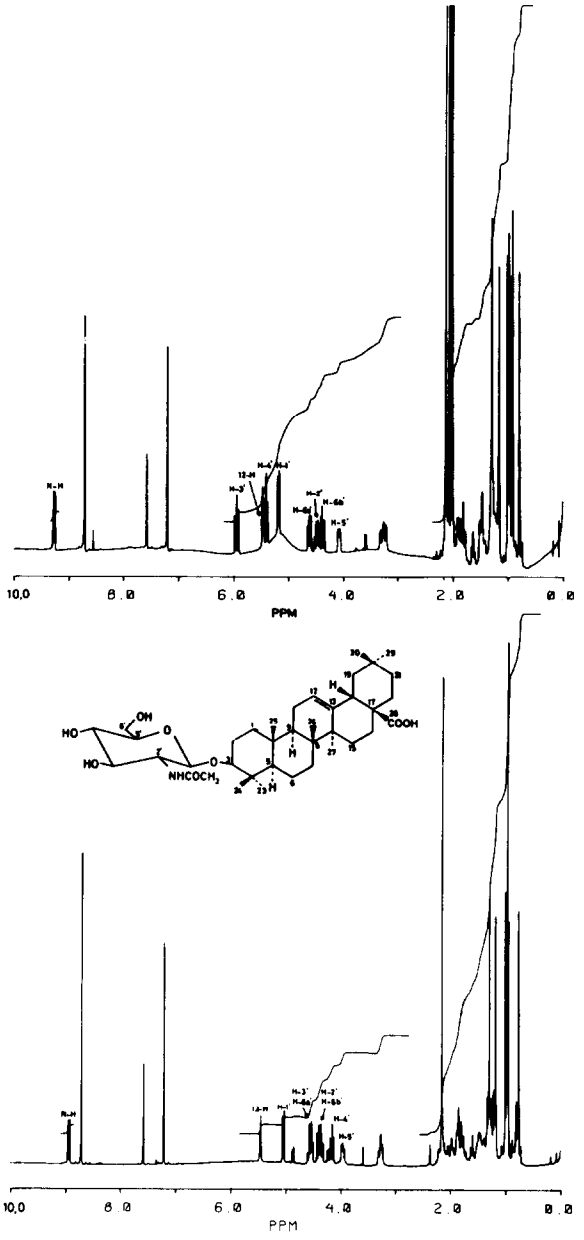


Fig. 1. Comparison of the ^{1}H NMR spectra of aridanin [3-*O*-(β-D-glucopyranosyl-2'-acetamido-2'-deoxy)-oleanolic acid] with that of its peracetylated derivative.

m/z 168 (45%) due to losses of two and three moles of acetic acid from the fragment at *m/z* 348. That aridanin contains a nitrogen atom is supported by CIMS peak matching experiments $C_{14}H_{20}NO_8$ for 330.118894 and $C_{14}H_{22}NO_9$ for 348.129459. Fragments **3** (*m/z* 207), **4** (*m/z* 248), **5** (*m/z* 203) and **7** (*m/z* 133) in the mass spectrum of aridanin and its peracetylated derivative are diagnostic of olean-12-enes and result from a retro-Diels–Alder reaction in ring C [8] further confirming the proposed structure for oleanolic acid.

From the above results, it was apparent that aridanin was composed of one molecule each of oleanolic acid and acetyl glucose. Its IR bands (KBr) at 1540, 1260, 1230 cm^{-1} (for –HNCO–), ^{1}H NMR signals and ^{13}C NMR signals at 168.73 (DMSO-d_6) clearly indicated the presence of an acetyl group. That the carbohydrate residue was linked with oleanolic acid through the hydroxyl at C-3 was clear from the mass spectrum of aridanin. EIMS afforded *m/z* (rel. int.): 641 $[659-H_2O]^+$ (0.06) and 456 [659 – acetylglucosyl]$^+$. The β-glycosidic linkage was indicated [9] by the large coupling constant of the anomeric proton in the ^{1}H NMR spectrum de-

Table 1. ^{13}C NMR spectral data for oleanolic acid, aridanin and peracetylated aridanin (22.6 MHz, δ-values, pyridine-d_5, TMS as int. standard)

Carbon	Oleanolic acid	Aridanin	Peracetylated Aridanin	Multiplicity (off-resonance ^{1}H-decoupled ^{13}C NMR of aridanin)
1	38.8	38.5	38.5	
2	27.9	26.2	26.1	
3	78.0	89.1	89.9	*d* (89.1)
4	39.3	39.1	39.1	
5	55.7	55.7	55.7	
6	18.7	18.4	18.5	
7	33.2	33.2	33.2	
8	39.6	39.6	39.7	
9	48.0	47.9	48.0	
10	37.3	36.9	36.9	
11	23.7	23.6	23.2†	
12	122.5	122.3	122.5	*d* (121.9)
13	144.7	144.7	144.8	*s* (144.8)
14	42.1	42.1	42.1	
15	28.2	28.1	28.1	
16	23.7	23.7	23.7	
17	46.6	46.6	46.6	
18	41.9	41.9	42.0	
19	46.4	46.4	46.6	
20	30.8	30.9	30.9	
21	34.1	34.1	34.2	
22	33.2	33.2	33.2	
23	28.2	28.1	28.1	
24	16.4	16.9	16.8	
25	15.4	15.4	15.3	
26	17.3	17.3	17.3	
27	26.1	26.1	26.1	
28	180.1	180.1	180.1	*s* (180.2)
29	33.2	33.2	33.2	
30	23.7	23.7	23.7	
1'	—	104.7	103.6	*d* (104.5)
2'	—	58.0	55.7	*d* (58.0)
3'	—	76.0	71.8*	*d* (76.1)
4'	—	72.6	70.1*	*d* (72.5)
5'	—	78.0	73.4*	*d* (78.1)
6'	—	62.9	62.8	*t* (62.9)
NAc/OAc	—	170.2	169.9	
			170.3	
			170.5	
			170.7	
NAc	—	23.6	23.7†	
OAc			20.6	
			20.5	
			21.0	

*,†Signals may be interchanged in pyridine-d_5.

scribed above. The position of the acetyl group was established by a consideration of the acetylation shifts [10, 11] of both the ^{1}H NMR and ^{13}C NMR signals (Fig. 1 and Table 1). The ^{1}H NMR spectrum of aridanin and the peracetylated derivative (Fig. 1) showed that the carbohydrate protons at C-3′ and C-4′ suffer shifts due to acetylation and the acetyl group can only be on C-2′ or C-6′. The results of the ^{13}C NMR spectrum (Table 1) showed that the acetamido group can only be on C-2′. This assignment agrees with published data for similar structures [12].

The structure of aridanin was thus established as 3-*O*-[β-D-glucopyranosyl-2′-acetamido-2′-deoxy]-oleanolic acid, a novel plant constituent.

EXPERIMENTAL

Mps are uncorr. IR spectra were taken as KBr pellets and specific rotations in pyridine. The UV spectra were recorded in MeOH. EIMS: AEI MS 12 and FDMS: Varian Instrument MAT 44s. ^{1}H NMR spectra were run at 60 MHz using TMS as internal standard. ^{13}C NMR measurements were achieved at 22.6 MHz on a Bruker WH 90. TLC (on silica gel layers) was carried out in solvent (a) toluene–EtOAc–HCOOH (5:4:1), (b) C_6H_6–EtOAc (8:2), (c) $CHCl_3$–EtOH (140:60), (d) EtOAc–MeOH–H_2O (10:2:1). TLC (on cellulose layers) was carried out in solvents (a), (b) or (e) C_6H_6–HOAc–H_2O (6:7:3, top layer). PC (on MN 261 paper) was carried out by the descending method in solvents (f) *n*-BuOH–HOAc–H_2O (4:1:5, top layer) and (g) *n*-BuOH–C_6H_6–pyridine–H_2O (5:1:3:3). Phenols, triterpenes and carbohydrates were revealed by standard procedures [13].

Plant material. The red-brown fruits of *T. tetraptera* Taub. were collected in Ile-Ife and were identified by the staff of the Forestry Research Institute of Nigeria who also keep an herbarium specimen.

Isolation and identification of compounds. Freshly collected fruit parts were ground into a fine powder (1 kg) and were successively extracted at room temp. with *n*-hexane (oily extractive, 3.4 g), $CHCl_3$ (7.5 g), 50% $CHCl_3$ in MeOH (204 g) and finally with MeOH (67 g). The early fractions were subjected to either TLC or PC for the isolation of hentriacontane ($C_{31}H_{64}$, 29 mg), mp 54°; IR ν_{max}^{KBr} cm^{-1}: 2960–2845, 1460, 1375, 725, 715, MS (*m/z*, rel. int.): 57 (100), 43 (70), 71 (60), 85 (38), 436 (2); scopoletin (72 mg), caffeic acid (5 mg) and *p*-coumaric acid (4 mg). The concd MeOH extract on long standing afforded a crystalline white residue (54 mg) identified by PC as a mixture of glucose, fructose and sucrose. The $CHCl_3$–MeOH extract (30 g) was chromatographed over silica gel G (Merck, 70–230 mesh) and eluted with $CHCl_3$–MeOH mixtures, the progress of the separation being monitored on silica gel G (TLC, solvent *d*). Aridanin (1.42 g, 0.142%) was eluted from the column with 11–12.5% MeOH in $CHCl_3$.

Aridanin ($C_{38}H_{61}NO_8$), white powder (MeOH), mp 276–280° (dec.); $[\alpha]_D^{25}$ + 40.96° (*c* 0.5; pyridine), R_fs 0.41, 0.59 (solvents c and d) was insoluble in H_2O, MeOH, $CHCl_3$ but soluble in $CHCl_3$–MeOH (1:1), pyridine and EtOH in dilute solns. IR ν_{max}^{KBr} cm^{-1}: 3500–3200 (OH), 2980–2860, 1690, 1660–1620, 1570–1540, 1460–1430, 1385, 1310, 1260, 1230, 1205, 1160, 1110, 1075–1021, 980, 890. EIMS *m/z* (rel. int.): 641 $[659-H_2O]^+$ (0.06), 613 $[659-H_2O-CO]^+$ (0.18), 568 (0.02), 456 [659 − acetylglucosyl]$^+$ (2), 439 (4.5), 423 (1.6), 395 (3.2), 300 (2.8), 248 (100), 203 (98), 189 (34), 133 (44), 69 (58), 55 (49). FDMS *m/z* (rel. int.): 659 $[M]^+$ (8), 660 $[M+1]^+$ (7), 614 $[660-H_2O-CO]^+$ (12), 456 (0.9), 439 (34), 304 (5.5), 262 (6), for $C_{38}H_{61}NO_8$, × H_2O. ^{1}H NMR (DMSO-d_6): δ1.76 (NAc), 5.16 (olefinic), 0.65, 0.71, 0.86, 0.87, 0.89, 1.07 (7 × Me). ^{13}C NMR (DMSO-d_6): δ178.55 (COOH), 168.75 (NAc), 143.84 (C-13), 121.52 (C-12). The carbohydrate carbon atoms appeared at 103.52, 55.88, 76.67, 74.04, 70.76 and 61.21, with NAc appearing at δ22.96. (Found: C, 66.2, H, 9.4, N, 1.8; Expected: C, 69.0, H, 9.2, N, 2.1%.) Peracetylated aridanin (Found: C, 65.5, H, 8.6, N (1.1); Expected: C, 67.2, H, 8.5, N, 1.8%).

Acid hydrolysis of aridanin. Aridanin (300 mg) was hydrolysed in 2% H_2SO_4–EtOH at 100° for 6 hr. On cooling, the white crystals that formed were filtered (113 mg). Further crystallization from aq. EtOH yielded a substance identified as oleanolic acid, mp 309–311° (lit. [14], 310–312°). IR ν_{max} cm^{-1}: 3480 (OH), 2960, 2880, 1700 (COOH), 1275. MS *m/z* (rel. int.): 456 (4), 249 (20), 248 (100), 203 (55); ^{1}H NMR ($CDCl_3$): δ5.27 (olefinic), 0.75, 0.77, 0.90, 0.91, 0.93, 0.96, 1.13 (Me × 7); ^{13}C NMR ($CDCl_3$): δ183.47 (COOH), 143.66 (C-13), 122.70 (C-12) (Table 1). Two derivatives viz acetyl oleanolate, mp 264–266°; IR ν_{max} cm^{-1}: 1745, 1700; MS *m/z* (rel. int.): 498 (0.7), 455 (0.6), 248 (100); ^{1}H NMR ($CDCl_3$): δ5.27 (H-12), 2.02 (OAc) and methyl oleanolate [mp 202–204°; IR ν_{max} cm^{-1}: 3480 (OH), 1735 (COOMe) 1170, 1140); MS *m/z* (rel. int.): 470 (5), 411 (2.2), 262 (70), 203 (100), ^{1}H NMR ($CDCl_3$): δ5.28 (H-12), 3.62 (COOMe)] were prepared and their analytical data compared with the literature data [14]. Neutralization of the acid hydrolysate in Amberlite IR-45 (OH^-) led to the identification of the carbohydrate derivative, white residue (6 mg), mp 148–154° dec.

Peracetylation of aridanin. To dry aridanin (120 mg) in dry pyridine (10 ml) was added Ac_2O (5 ml) and acetylation was allowed to progress at room temp. during 24 hr. After the usual work-up procedure, the product (123 mg), white powder, was recovered, mp 261–265° (dec.); IR ν_{max} cm^{-1}: 2945, 1750–1735 (br. acetate), 1385, 1230, 1040 (C–O–C), 985, 910. EIMS *m/z* (rel. int.): 784 $[785-H]^+$ (0.13), 741 $[785-43-H]^+$ (0.02), 538 (7), 438 (28), 391 (21), 348 (50), 330 (85), 270 (11), 248 (90), 203 (100), 210 (56), 190 (96), 168 (45), 189 (52), 150 (88), 133 (40). FDMS *m/z* (rel. int.): 785 $[M]^+$ (18), 786 $[M+1]^+$ (100), 757 $[785-CO]^+$ (100), 739 $[757-28]^+$ (100), 393 (33). ^{1}H NMR ($CDCl_3$, 300 MHz): δ1.92 (NAc), 2.01, 2.04, 2.06 (3 × OAc), 0.73 (2 × Me), 0.90 (3 × Me), 0.92 (Me), 1.10 (Me). The carbohydrate protons appeared at δ3.6–5.5 with the anomeric proton at δ4.77 (*d*, *J* = 9.8 Hz, cf. Fig. 1). ^{13}C NMR ($CDCl_3$): δ184.30 (COOH), 143.58 (C-13), 122.62 (C-12), 169.48 (NAc), 170.85, 170.68, 170.62 (3 × OAc). The carbohydrate carbons appeared at 102.83 (anomeric), 55.39, 72.06, 71.28, 69.30 and 62.57 with NAc appearing at 23.09.

Acknowledgements—We express our gratitute to Professor Dr. Thiem and Frau Sabina Kopper (Institute of Organic Chemistry, Muenster) for their helpful contributions on the position of the acetamido group, to Deutsche Forschungsgemeinschaft for financial support and to Heinrich Hertz Stiftung for a Fellowship (S.K.A.).

REFERENCES

1. Adesina, S. K., Adewunmi, C. O. and Marquis, V. O. (1980) *J. Afr. Med. Plants* **3**, 7.
2. Gmelin, R. and Larsen, P. O. (1967) *Biochim. Biophys. Acta* **136**, 572.
3. Krauss, G. Y. J. and Reinbothe, H. (1973) *Phytochemistry* **12**, 125.
4. Ojewole, J. A. O. and Adesina, S. K. (1983) *Planta Med.* **49**, 46.
5. Ojewole, J. A. O. and Adesina, S. K. (1983) *Planta Med.* **49**, 99.
6. Hanessian, S. (1972) *Methods Carbohydr. Chem.* **6**, 208.

7. Hogg, A. M. and Nagabhushan, T. L. (1972) *Tetrahedron Letters* 4827.
8. Budzikiewicz, H., Wilson, J. M. and Djerassi, C. (1963) *J. Am. Chem. Soc.* **85**, 3688.
9. Otsuka, H., Akiyama, T., Kawai, K., Shibata, S., Inoue, O. and Ogihara, Y. (1978) *Phytochemistry* **17**, 1349.
10. Tori, K., Seo, S., Shimaoka, A. and Tomita, Y. (1974) *Tetrahedron Letters* **48**, 4227.
11. Ishii, H., Nakamura, M., Seo, S., Tori, K., Tozyo, T. and Yoshimura, Y. (1980) *Chem. Pharm. Bull.* **28**, 2367.
12. Bundle, D. R., Jennings, H. J. and Smith, I. C. P. (1973) *Can. J. Chem.* **51**, 3812.
13. Harborne, J. B. (1973) *Phytochemical Methods*. Chapman & Hall, London.
14. West, L. G. and McLaughin, J. L. (1977) *Lloydia* **40**, 499.