PERGAMON

Phytochemistry 51 (1999) 927–930

PHYTOCHEMISTRY

Limonoids from *Aphanamixis polystacha*

Dulcie A. Mulholland*, Nesan Naidoo

Natural Products Research Group, Department of Chemistry, University of Natal, Durban, 4041, South Africa

Received 25 January 1999; accepted 8 March 1999

Abstract

The hexane extract of the seed of *Aphanamixis polystacha* (Meliaceae) has yielded kihadalactone A and three novel limonoids, rohituka-12, rohituka-13 and rohituka-14. The substitution at C-1 of polystachin has been re-assigned as α. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Aphanamixis polystacha*; Meliaceae; Polystachin; Limonoids; Rohituka-12; Rohituka-13 and Rohituka-14; Kihadalactone A

1. Introduction

Aphanamixis polystacha (Wall) J.N. Barker is an Indo-Malaysian member of the Meliaceae. This plant has been extensively investigated and has yielded the labdane diterpenoid, aphanamixol, from the fruit (Chandrasekharan & Chakraborty, 1968) and leaves (Monkhe, 1991), the protolimonoids turraeanthin, aphanamixin and aphananin (Chatterjee, Kundu, Chandrasekharan & Chakraborty, 1970; Kundu, Sanghamitra & Chatterjee, 1985) from the seed, and a range of complex limonoids including rohitukin, polystachin, rohituka 1–7, prieurianin and hispidin C from the seed (Mulholland & Taylor, 1979; Jolad et al., 1981; Brown & Taylor, 1978; Gullo et al., 1975; Connolly, Okorie, de Wit & Taylor, 1976; MacLachlan & Taylor, 1982).

The seed of this species was re-investigated firstly to re-isolate polystachin in order to confirm the stereochemistry at C-1, secondly, to assign the ^{13}C NMR spectrum of polystachin and thirdly to isolate more novel compounds from the complex mixture of limonoids produced by the seed of this species. In compounds such as methyl ivorensate and ekebergin (Adesogan & Taylor, 1970; Taylor, 1981) the 1,14-oxide stereochemistry has been established as 1α,14β. However, the stereochemistry for polystachin was assigned as 1β,14β because $W_{1/2}$ for H-1 of polystachin (16 Hz) differed from that of methyl ivorensate (10 Hz) (Mulholland & Taylor, 1979). Seed was collected from a tree growing on the University of Natal Durban campus and a voucher specimen retained.

2. Discussion

The hexane extract of *A. polystacha* was separated using column chromatography over silica gel and yielded the known compounds polystachin, **1**, rohituka-7 and rohituka-9, which have been isolated previously from this source, kihadalactone A which has been isolated previously from *Phellodendron amurense* (Meliaceae) (Kishi, Yoshikawa & Arihara, 1992) and the novel limonoids rohituka 12(**2**), 13(**3**), and 14(**4**). The methanol extract yielded a crystalline material which was identified as sucrose, but no limonoids. The isolation of the ring A-lactone limonoid kihadalactone is the first report of a relatively simple limonoid from *A. polystacha* and is of interest as the complex rohitukin limonoids would arise from compounds of this type by oxidation of ring B and the Δ^{14}-double bond.

NOE difference and 2D NMR experiments on polystachin enabled assignment of the ^{1}H and ^{13}C NMR resonances to be performed (Tables 1 and 2). A posi-

---

* Corresponding author.

E-mail address: mulholld@scifs1.und.ac.za (D.A. Mulholland)

PII: S0031-9422(99)00157-0

Table 1
^{1}H NMR data for **1**, **2**, **3** and **4** ($CDCl_3$, 300 MHz, *J* in parenthesis)

H	**1**	**2**	**3**	**4**
1	3.79*t* (9.3)	3.69*dd* (7.2, 10.4)	4.07 *m*	4.01 *m*
2	3.18 *m*, 2.96 *m*	3.13 *m*, 2.85 *m*	3.11 *m*, 2.65 *m*	3.10 *m*, 2.77 *m*
5	2.91 *m*	2.89 *m*	2.74 *m*	2.32 *m*
6	2.55 *m*, 2.29 *m*	2.58 *m*, 2.31 *m*	2.73 *m*, 2.33 *m*	2.73 *m*, 2.33 *m*
9	2.70 *d* (5.4)	2.70 *d* (5.7)	3.16 *d* (9.9)	3.19 *d* (9.9)
11	5.32*dd* (5.4, 10.8)	3.96 *m*	4.07 *m*	4.13 *m*
12	6.03 *d* (10.8)	5.81 *d* (9.9)	5.74 *d* (8.1)	5.75 *d* (7.8)
16	2.84 *m*, 2.36 *m*	2.78 *m*, 2.32 *m*	2.33 *m*, 1.95 *m*	2.77 *m*, 2.34 *m*
17	3.86 *m*	3.85 *m*	3.77 *m*	3.78 *m*
18	0.88 *s*	0.79 *s*	0.81 *s*	0.84 *s*
19	1.02 *s*	1.10 *s*	1.16 *s*	1.14 *s*
21	7.23 *s*	7.22 *s*	7.13 *s*	7.20 *s*
22	6.20 *s*	6.21 *s*	6.22 *s*	6.21 *s*
23	7.33 *s*	7.33 *s*	7.26 *s*	7.33 *s*
28	1.98 *s*	1.83 *s*	1.65 *s*	1.65 *s*
29	4.18 *d* (12.0)	4.21 *d* (11.7)	4.21 *d* (11.4)	4.23 *d*(11.1)
	4.08 *d* (11.7)	3.99 *d*(11.7)	3.98 *d*(11.4)	3.98 *d*(11.4)
30	5.47 *s*, 5.47 *s*	5.36 *s*, 5.34 *s*	5.13 *s*, 5.05 *s*	6.21 *s*, 5.20 *s*
2′	1.70 *m*	1.70	1.70 *m*	1.82 *m*
3′	1.65 *m*	1.65	1.65 *m*	1.82 *m*
4′	0.75 *d*	0.75 *d*	0.75 *d*	0.90 *d*
5′	0.85 *d*	0.85 *d*	0.85 *d*	0.88 *d*
1″	8.11 *s*	–	–	–

Table 2
^{13}C NMR data for **1**, **2**, **3** and **4** ($CDCl_3$, 75 MHz)

C	**1**	**2**	**3**	**4**
1	73.6 *d*	74.3 *d*	78.1 *d*	78.3 *d*
2	38.3*t*	38.7*t*	37.0*t*	37.1*t*
3	172.3 *s*	173.8 *s*	172.6 *s*[a]	172.7 *s*[a]
4	78.6 *s*	79.4 *s*	79.4 *s*	79.5 *s*
5	40.7 *d*	41.2 *d*	43.8 *d*	43.7 *d*
6	32.7*t*	32.4*t*	31.3*t*	31.2*t*
7	172.3 *s*	172.6 *s*	171.4 *s*[a]	171.4 *s*[a]
8	134.5 *s*	135.9 *s*	140.3 *s*	139.1 *s*
9	55.3 *d*	56.1 *d*	53.8 *d*	54.6 *d*
10	49.9 *s*[a]	50.7 *s*[a]	49.5 *d*	48.1 *s*
11	72.5 *d*	74.9 *d*	79.6 *d*	79.6 *d*
12	72.8 *d*	77.5 *d*	73.6 *d*	73.2 *d*
13	48.9 *s*[a]	48.9 *s*[a]	51.8 *s*	51.7 *s*
14	87.4 *s*	87.4 *s*	84.0 *s*	79.5 *s*
15	205.3 *s*	205.9 *s*	74.2 *d*	209.3 *s*
16	41.1*t*	41.2*t*	37.0*t*	42.0*t*
17	36.8 *d*	37.0 *d*	38.8 *d*	34.8 *d*
18	12.2*q*	15.5*q*	13.3*q*	13.0*q*
19	22.2*q*	22.7*q*	16.0*q*	16.6*q*
20	121.7 *s*	122.3 *s*	123.9 *s*	122.7 *s*
21	140.6 *d*	140.7 *d*	142.4 *d*	140.5 *d*
22	110.4 *d*	110.6 *s*	110.8 *s*	110.5 *s*
23	142.9 *d*	142.9 *d*	143.1 *d*	142.7 *d*
28	29.1*q*	28.2*q*	26.6*q*	26.7*q*
29	74.3*t*	73.9*t*	72.3*t*	72.7*t*
30	118.9*t*	117.7 *s*	117.0 *s*	120.9 *s*
1′	167.6 *s*	168.2 *s*	167.5 *s*	167.6 *s*
2′	24.8 *d*	43.1*t*	24.8 *d*	43.2*t*
3′	43.0 *d*	24.9 *d*	93.0*t*	25.24 *d*
4′	22.4*q*	22.4*q*	22.4*q*	22.4*q*
5′	22.3*q*	22.5*q*	22.3*q*	22.3*q*
1″	160.2 *d*		–	–
CH_3**C**OO			169.5 *s*	–
CH_3COO			21.2*q*	–

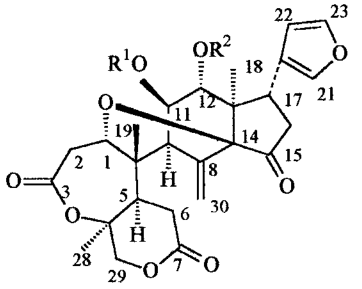
[a] Values in column may be interchanged.

tive NOE was shown between H-1 (δ 3.79) and the 3H-19 methyl group protons. The C-19 methyl group is known to be in the β-orientation thus H-1 would have to be β. Thus the original assignment of the stereochemistry (Mulholland & Taylor, 1979) was incorrect and the structure of polystachin is re-assigned as **1** with the 1α,14β-oxide bridge as found in other limonoids of this type.

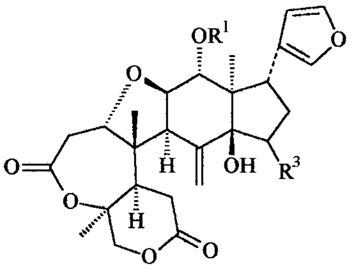
Mass spectrometry of rohituka-12 indicated a molecular formula of $C_{31}H_{38}O_{10}$. A peak at $[M-18]^+$ indicated the loss of a water molecule, indicating the presence of a hydroxy group in the molecule. This was confirmed by the IR spectrum. The NMR spectra of rohituka-12 were similar to those for polystachin but lacked the resonances due to the formate group at C-11β present in the NMR spectra of polystachin. COSY and HETCOR spectra indicated that H-11α resonated at δ 3.96 (*dd*) and C-11 at δ 74.9. Thus the hydroxy group was placed at C-11β instead of the formate. Thus structure **2** was assigned to rohitukin 12.

Rohituka 13, $C_{33}H_{42}O_{11}$, was found to be similar to the previously isolated rohituka 7, **5**. Ring D had a hydroxy group at C-14β and an acetate group at C-15β. Ring B was opened and recyclised to give a 7, 29-lactone, an ester was present at C-12α and H-12β occurred as a doublet with $J = 7$ Hz as in **5** indicating substitution at C-11β. The stereochemistry at these positions was confirmed for rohituka 7 by X-ray analysis (King & Taylor, 1983). However, the NMR spectra lacked resonances ascribable to H-1 and H-2 of the α, β-unsaturated ring A lactone and the formate group at C-11β. The COSY spectrum showed that a new resonance ascribable to H-1 at δ4.07 was coupled to the non-equivalent H-2 protons at δ3.11 and δ2.80. The HETCOR spectrum showed the resonance at δ4.07 correlated with a resonance at δ78.1, indicating an oxygen atom at C-1. H-11α occurred as a double doublet at δ4.07 and the COSY spectrum indicated coupling to H-9 and H-12β. The HETCOR spectrum showed that C-11 occurred at δ79.6 indicating an oxygen atom at C-11. The molecular formula indicated another ring was required and that only one oxygen atom was available to be attached to both C-1 and C-11, indicating an ether linkage between C-1 and C-11 as in rohitukin (Connolly et al., 1976). Thus structure **3** was assigned to rohituka 13.

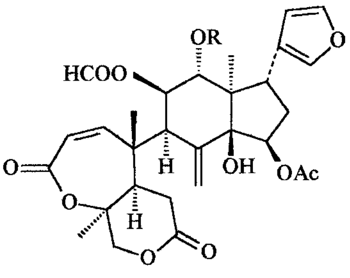
Rohituka 14, $C_{31}H_{38}O_{10}$, differed from rohituka 13 in that the resonances ascribed to H-15 and C-15 were no longer present in the NMR spectra, but an extra resonance occurred at δ209.3 indicating a keto group at C-15. Thus structure **4** was assigned to rohituka 14.



	R¹	R²
1	HCO	$(CH_3)_2CH\,CH_2CO$
2	H	$(CH_3)_2CH\,CH_2CO$



	R¹	R³
3	$(CH_3)_2CH\,CH_2CO$	βOAc
4	$(CH_3)_2CH\,CH_2CO$	=O



5 R = $COCH(OH)CH(CH_3)CH_2CH_3$

3. Experimental

3.1. General

Minced seed (605 g) of *A. polystacha* was extracted in a soxhlet apparatus with refluxing hexane yielding, after evaporation, an oily extract. The extract was extracted with aqueous methanol (3 × 50 ml, 90%), the methanolic extract was diluted with water to give a 50:50, methanol:water mixture and extracted with chloroform to give, after evaporation of the chloroform, a gummy extract (23 g). Repeated CC over silica gel (Merck 9385) yielded seven limonoids, the known polystachin, **1** (6.4 mg), rohituka 7 (3.2 mg) rohituka 9 (3.3 mg) and kihadalactone A (2.0 mg) whose structures were determined by comparison with literature data (Mulholland & Taylor, 1979; Brown & Taylor, 1978; Kishi, Yoshikawa & Arihara, 1992) and the novel limonoids, rohituka 12, **2**, rohituka 13, **3** and rohituka 14, **4**. ^{1}H and ^{13}C NMR data for **1**, **2**, **3** and **4** are given in tables 1 and 2.

NMR spectra were recorded in $CDCl_3$, with TMS as an int. standard. FAB MS were recorded by Dr P. Boshoff of the Cape Technikon.

3.2. Polystachin (1)

(6.4 mg), amorphous. FAB MS $[MNa]^+$ at *m/z* 621, 599 $[MH]^+$, 391, 363, 341. IR: ν_{max} (NaCl) cm^{-1}: 2961, 1754, 1474, 1383, 1151, 1081, 758. $[\alpha]_D = -74.3°$ ($CHCl_3$, c 3.8×10^{-4}).

3.3. Rohitukin 12 (2)

(4.4 mg), amorphous, FAB MS $[MNa]^+$ at *m/z* 593, 571 $[MH]^+$, 553 $[MH–H_2O]^+$, 469 $[MH–C_5H_{10}O_2]^+$, 451 $[MH–C_5H_{10}O_2–H_2O]^+$, 413, 391, 357, 339. IR: ν_{max} (NaCl) cm^{-1}: 3413, 2960, 1745, 1463, 1291 $[\alpha]_D = -79.6°$ ($CHCl_3$, c 4.4×10^{-4}).

3.4. Rohituka 13 (3)

(3.7 mg), amorphous, FAB MS $[MH]^+$ at *m/z* 615, 453 $[MH–CH_5COOH–C_5H_{10}O_2]^+$. IR: ν_{max} (NaCl) cm^{-1}: 2959, 2926, 1742, 1465, 1371, 1075, 756. $[\alpha]_D = -45.1°$ ($CHCl_3$, $c = 6.6 \times 10^{-4}$).

3.5. Rohituka 14 (4)

(2.3 mg), amorphous, FAB MS $[MH]^+$ at *m/z* 571, 553 $[MH–H_2O]^+$, 469 $[MH–C_5H_{10}O_2]^+$, 451 $[MH–C_5H_{10}O_2–H_2O]^+$, 413, 391, 357, 339. IR: ν_{max} (NaCl) cm^{-1}: 2960, 2931, 1728, 1463, 743.

Acknowledgements

This research was funded by the University of Natal Research Fund. N. Naidoo gratefully acknowledges a Foundation for Research Development Postgraduate Bursary and Dr P. Boshoff for providing mass spectra.

References

Adesogan E.K., Taylor D.A.H. (1970). Limonoid extractives from *Khaya ivorensis. J.C.S.(C)*, 1710.

Brown, D. A., & Taylor, D. A. H. (1978). Limonoid extractives from *Aphanamixis polystacha. Phytochemistry*, *17*, 1995.

Chandrasekharan, S., & Chakraborty, T. (1968). Aphanamixol, a diterpene alcohol from *Aphanamixis polystacha* (wall) Parker. *J. Indian Chem. Soc.*, *45*, 208.

Chatterjee, A., Kundu, A. B., Chandrasekharan, S., & Chakraborty, T. (1970). *Tetrahedron*, *26*, 1859.

Connolly J.D., Okorie D.A., de Wit D.L., Taylor D.A.H. (1976). Structure of Dregeanin and Rohitukin. *Chem. Comm.*, 909.

Gullo V.P., Miura T., Nakanishi K., Cameron A.F., Connolly J.D., Duncanson F.D., Harding A.E., McCrindle R., Taylor D.A.H. (1975). Structure of prieuranin, a complex tetranortriterpenoid. *J. Chem. Soc. Chem. Commun.*, 345.

Jolad, S., Hoffmann, J., Schram, K., Cole, J., Tempesta, M., & Bates, R. (1981). Constituents of *Trichilia hispida* (Meliaceae). *J.O.C.*, *46*, 641.

King, T. J., & Taylor, D. A. H. (1983). Limonoids from *Aphanamixis polystacha. Phytochemistry*, *22*, 307.

Kishi, K., Yoshikawa, K., & Arihara, S. (1992). Limonoids from the fruit of *Phellodendron amurense. Phytochemistry*, *31*, 1335.

Kundu, A. B., Sanghamitra, R., & Chatterjee, A. (1985). Aphananin, a triterpene from *Aphanamixis polystacha. Phytochemistry*, *24*, 2123.

MacLachlan, L., & Taylor, D. A. H. (1982). A revision of the structures of three limonoids. *Phytochemistry*, *21*, 2426.

Monkhe T.V. Extractives from the meliaceae. MSc dissertation University of Natal, Durban (1991).

Mulholland D.A., Taylor D.A.H. (1979). A new limonoid from *Aphanamixis polystacha. J. Chem. Res. Synop.*, 294.

Taylor, D. A. H. (1981). Ekebergin, a limonoid extractive from *Ekebergia capensis. Phytochemistry*, *20*, 2263.