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NEW PROTOLIMONOIDS FROM THE FRUITS OF *PHELLODENDRON CHINENSE**

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Key Word Index—*Phellodendron chinense*; Rutaceae; tirucallane euphane triterpenes; protolimonoids; niloticin; niloticin acetate; dihydroniloticin.

Abstract—Six tetracyclic triterpenes based on the tirucallane/euphane skeleton have been isolated from the fruits of *Phellodendron chinense*. Three of the triterpenes appear to be novel and have been characterized as niloticin (23ξ-hydroxy-3-oxo-20ξ-tirrucalla-7-en-24ξ, 25-oxide), niloticin acetate and dihydroniloticin (3β, 23ξ-dihydroxy-20-ξ-tirrucalla-7-en-24ξ,25-oxide). All exhibit oxygenation at C-23, C-24 and C-25 indicative of the initial stages in the formation of the C-17 furanoid system of limonoids.

INTRODUCTION

Phellodendron chinense Schneider can probably be considered as part of the *Phellodendron amurense* Rupr. aggregate. This taxon, which is found throughout temperate China, Japan and the eastern U.S.S.R. is a large tree characterized by its extremely corky bark. Previous studies have led to the isolation of benzyltetrahydroisoquinoline-derived alkaloids [1], prenylated flavonoids [2] and limonoids [3].

In the course of our studies on the Rutaceae we have examined the ripe fruits from *P. chinense* growing in the National Botanic Gardens, Dublin. These have yielded a number of triterpenes based on the tirucallane/euphane skeleton. In this paper we report on the identification of six compounds (**1**–**6**) in which the C-17 side-chain has undergone oxidation but has not cyclized. Three of these compounds are known, cneorin-NP$_{36}$ (**1**) [4], piscidinol-A (**5**) [5] and hispidol-B (**6**) [6]; the other three appear to be novel.

RESULTS AND DISCUSSION

Column chromatography of a concentrate from the petrol (bp 40–60°) extract eluting with petrol containing increasing amounts of ethyl acetate gave **1** to **6**, in order of elution.

The least polar compound analysed for $C_{30}H_{46}O_3$. The high-resolution ^{1}H NMR spectrum (Table 1) showed complete agreement with that recorded for **1** [4] and the ^{13}C NMR spectrum (Table 2), reported here for the first time, is in agreement with that anticipated for **1**. The stereochemistry depicted in structure **1** follows that indicated by Mondon *et al.* [4] but it should be noted that the evidence for this seems far from conclusive.

The next three compounds to be eluted were related to each other by acetylation (**3**→**2**) and by reduction (**3**→**4**). The major compound (**3**) analysed for $C_{30}H_{48}O_3$ with the IR spectrum indicating the presence of OH and C=O functional groups. The ^{1}H NMR spectrum (Table 1) showed clear resonances for C-2 protons in a 3-oxo triterpene (cf. **1**) and a major fragment at *m/z* 325 (**7**) confirmed that the tetracyclic part of the structure was comparable to **1**. A further feature of the EIMS was a significant ion at *m/z* 71 (**8**) which suggested a 24,25-epoxide (again cf. **1**). The ^{1}H NMR spectrum revealed a typical epoxide oxymethine proton as a doublet at δ 2.64 which decoupling experiments showed to be coupled to an oxymethine proton appearing at δ 3.49 as a doublet of doublets of doublets. On this basis the structure of the major compound must be established as **3** and **2** must be the corresponding 23-acetate in which the H-23 proton is deshielded to δ 4.86. Reduction of **3** with sodium borohydride gave the 3β,23-diol (**4**), identical in all respects to the isolated compound. Detailed ^{1}H NMR data for **2**–**4** are recorded in Table 1 and ^{13}C NMR data for **3** and **4** in Table 2. At the time of submission of this paper all three compounds appeared to be novel, although **3** had previously been synthesized [7]. However, after acceptance of this paper we became aware (T. Smith, personal communication) that **3** and **4** have recently been isolated from *Turrea nilotica* (Meliaceae) [8] and we have adopted the trivial names employed therein.

Oxidation of the diacetate (**9**) with mercuric acetate and HOAc yielded the anticipated heteroannular 7,9(11)-diene (**10**) [9], the ORD spectrum of which exhibited the anticipated negative Cotton effect. This is typical of euphane/tirucallane type skeletons and contrary to the strong positive Cotton effect exhibited by lanost-7,9(11)-dienes [10]. Stereochemistry for C-20, C-23 and C-24 is unassigned but it seems likely that these compounds

* Most of the work reported here was undertaken when A.I.G. and P.B. were at the Department of Pharmacognosy, School of Pharmacy, Trinity College Dublin, Ireland.

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Table 1. ^{1}H NMR assignments for **1–6**

Proton	1	2	3	4	5	6
H-1$_{eq}$	1.98 *ddd* (13.3,5.5,3.3)		2.02 *m*		1.97 *ddd* (13.3,5.7,3.2)	
H-1$_{ax}$	1.48 *m*		1.40 *m*			
H-2$_{eq}$	2.21 *dt* (14.5,3.3)	2.27 *m*	2.17 *m*		2.23 *dt* (14.6,3.2)	
H-2$_{ax}$	2.73 *td* (14.5,5.5)	2.75 *td* (14.5,5.5)	2.68*td* (14.0,5.5)		2.74 *td* (14.6,5.7)	
H-3$_{ax}$				3.25 *dd* (10.0,5.0)		3.50 *m*
2H-6	2.06/2.25	2.08/2.27	2.17/2.02	2.00–2.30	2.23/2.10	
H-7	5.30 *td* (3.3,3.1)	5.31 *td* (3.3,3.1)	5.27 *td* (3.5,3.0)	5.27 *td* (3.2,3.0)	5.30 *td* (3.3,3.0)	5.32 *br s*
H-9	2.06 *m*		2.02 *m*	2.00–2.30	2.10 *m*	
H-20	1.25 *m*					
H-22	2.61 *dd* (8.1,2.2)		1.40/1.55 *m*		1.25 *m*	
H-23	2.78 *dd* (6.1,2.2)	4.86 *td* (8.5,5.3)	3.49 *dtd* (8.4,5.2,2.7)	3.57 *td* (8.2,5.0)	4.10 *td* (8.7,4.1)	4.64 *m*
H-24	2.51 *d* (6.1)	2.75 *d* (8.5)	2.64 *d* (8.4)	2.66 *d* (8.2)	3.15 *d* (8.7)	3.72 *d* (8.0)
Me-18	0.99 *s*	1.00 *s*	0.94 *s*	0.81 *s*	0.99 *s*	0.91 *s*
Me-19	0.77 *s*	0.80 *s*	0.75 *s*	0.75 *s*	0.81 *s*	0.83 *s*
Me-21	1.03 *d* (6.0)	0.95 *d* (6.2)	0.89 *d* (6.0)	0.95 *d* (6.2)	0.91 *d* (6.3)	1.16 *d* (6.1)
Me-26/	1.39 *s*	1.35 *s*	1.25 *s*	1.34 *s*	1.31 *s*	1.68 *s*
27	1.32 *s*	1.32 *s*	1.26 *s*	1.32 *s*	1.29 *s*	1.66 *s*
Me-28	1.09 *s*	1.10 *s*	1.05 *s*	0.99 *s*	1.10 *s*	1.18 *s*
Me-29	1.02 *s*	1.00 *s*	0.95 *s*	0.86 *s*	1.00 *s*	1.02 *s*
Me-30	1.02 *s*	1.04 *s*	0.95 *s*	0.97 *s*	1.03 *s*	1.14 *s*
O-H			2.58 *d* (2.7)		2.62 *d* (8.7) 2.58 *d* (4.1) 2.57 *s*	
OAc		2.05 *s*				

All spectra run in $CDCl_3$ except for **6** which was obtained in pyridine-d_5

belong to the 20*S* (H-20α) tirucallane series which is common in the Meliaceae and also occurs in the Rutaceae [3].

The penultimate compound analysed for $C_{30}H_{50}O_4$ and gave spectral data in agreement with that published [5] for the 3-oxo-23,24,25-triol piscidinol A (**5**) which has previously been isolated from *Walsura piscidia* Roxb. (Meliaceae). The final compound was identified as hispidol-B (**6**) which has previously been isolated [6] from another meliaceous plant, *Trichilia hispida* Pennington.

The six isolated triterpenes all exhibit considerable oxidation of the C-17 side-chain. This is the first time any of these compounds have been recorded from the Rutaceae but the occurrence of **1** in the Cneoraceae and **3–6** in the Meliaceae is indicative of the existence of similar oxidation processes, coincident with the initial stages of limonoid biosynthesis, in all three families. However, one notable feature lacking is oxidation of the 21-Me group which is a prerequisite for formation of the furanoid 21,23-oxide system typical of limonoids.

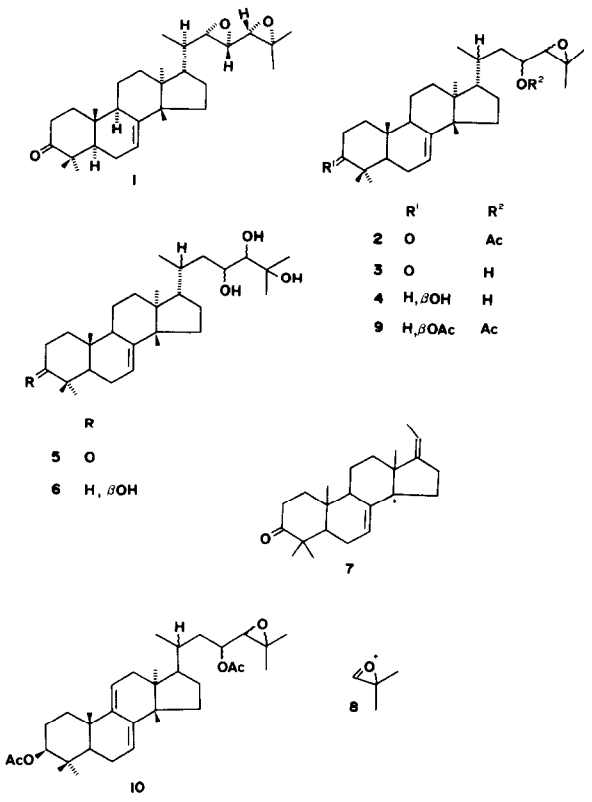
EXPERIMENTAL

Mps uncorr. UV: EtOH, IR: KBr discs. ^{1}H and ^{13}C NMR run in $CDCl_3$ using TMS as int. standard; field-strengths as noted in the text. EIMS obtained at 70 eV using direct probe insert and elevated temp.

Plant material. Fruit of *Phellodendron chinense* were collected in the National Botanic Gardens, Glasnevin, Dublin in August 1984. A voucher specimen has been deposited at the Herbarium of the Botanic Garden.

Extraction and isolation of compounds The dried, powdered, fruits (800 g) were extracted with petrol and the resulting extract concd. to give a solid (20 g) which was subjected to CC over silica gel. Elution with petrol containing increasing amounts of EtOAc gave, in order of elution, **1** (38 mg), **2** (55 mg), **3** (2.6 g), **4** (410 mg), **5** (360 mg) and **6** (48 mg).

Cneorin-NP$_{36}$ (**1**). Needles mp 179–180° (Lit. [4] 178°), $[\alpha]_D$ −97° ($CHCl_3$; *c* 0.14) (Lit. [4] −86°). Found: M^+ 474.3440; $C_{30}H_{46}O_3$ requires 474.3447. IR ν_{max} cm^{-1}: 2965, 2950, 2880, 1715, 1460, 1388, 900, 890, 820. ^{1}H NMR see Table 1. ^{13}C NMR



see Table 2. EIMS *m/z* (rel. int.): 474 $[M]^+$ (100), 439 (98), 381 (15), 363 (11), 337 (11), 325 $[C_{23}H_{33}O]^+$ (34), 297 $[C_{21}H_{29}O]^+$ (18), 271 (12), 159 (18), 133 (27), 125 (24), 113 $[C_6H_9O_2]^+$ (63), 95 (50), 71 (25).

Niloticin acetate (**2**). Needles mp 157°; $[\alpha]_D$ −75° ($CHCl_3$; *c* 0.035). Found: M^+ 498.3729; $C_{32}H_{50}O_4$ requires 498.3709. IR ν_{max} cm^{-1}: 2980, 2960, 2880, 2855, 1738, 1710, 1380, 1243, 1123, 1025, 964, 918, 838, 792. ^1H NMR see Table 1. EIMS *m/z* (rel. int.): 498 $[M]^+$ (57), 483 (53), 438 (1), 423 (21), 405 (8), 369 (10), 365 (9), 351 (12), 325 (59), 313 (5), 297 (7), 271 (15), 257 (6), 187 (9), 173 (7), 159 (11), 147 (14), 125 (20), 107 (28), 97 (16), 95 (34), 71 (20), 59 (12), 43 (100).

Niloticin (**3**). Needles, mp 147°, $[\alpha]_D$ −62° ($CHCl_3$; *c* 0.08). Found: M^+ 456.3596; $C_{30}H_{48}O_3$ requires 456.3603. IR ν_{max} cm^{-1}: 3470, 2960, 1709, 1460, 1387, 1293, 1120, 1062, 970, 880, 840, 815. ^1H NMR see Table 1. ^{13}C NMR see Table 2. EIMS *m/z* (rel. int.): 456 $[M]^+$ (41), 441 (10), 384 (14), 369 $[C_{25}H_{37}O_2]^+$ (100), 351 (17), 325 (44), 271 (11), 257 (4), 147 (13), 133 (18), 125 (13), 71 (11). ORD ($CHCl_3$) $[\phi]_{315nm}$-3028, $[\phi]_{283nm}$ 0, $[\phi]_{275nm}$ +228, $[\phi]_{267nm}$ 0. Acetylation of **3** (30 mg) dissolved in pyridine (0.5 ml) and Ac_2O (0.5 ml) overnight at room temp followed by normal work-up gave a product identical in all respects to **2**. Reduction of **3** (80 mg) in MeOH (6 ml) was achieved by stirring with $NaBH_4$ (25 mg) for 45 min. The reaction mixture was diluted with H_2O, neutralized and extracted into $CHCl_3$. The residue was identical in all respects to **4**.

Dihydroniloticin (**4**). Needles from MeOH, mp 174°, $[\alpha]_D$ −47° ($CHCl_3$; *c* 0.075). Found: M^+ 458.3771; $C_{30}H_{50}O_3$ requires 458.3771. IR ν_{max} cm^{-1}: 3410, 2950, 2925, 1455, 1380, 1030, 905. ^1H NMR see Table 1. ^{13}C NMR see Table 2. EIMS *m/z* (rel. int.): 458 $[M]^+$ (63), 443 (14), 425 (6), 407 (1), 386 (13), 371 (100), 353 (49), 327 (26), 273 (4), 255 (2), 147 (20), 135 (31), 133 (23), 71 (21).

Table 2. ^{13}C NMR assignments for **1**, **3**, **4** and **6** -(3,23,24-triacetate)

C	1	3	4	6-$(OAc)_3$[6]
1	38.4	38.1	37.3	36.8
2	34.7	34.4	28.8	24.2
3	216.4	215.8	79.3	81.2
4	47.7	47.4	39.0	37.9
5	52.2	52.0	50.7	50.8
6	24.3	24.0	24.0	23.8
7	118.1	117.6	118.1	117.8
8	145.3	145.3	145.6	145.7
9	48.3	48.1	49.1	48.8
10	34.9	34.6	35.0	34.8
11	18.0	17.9	18.2	18.1
12	33.2	33.3	34.1	33.2
13	43.8	43.2	43.7	43.6
14	50.7	50.8	51.2	51.1
15	34.1	33.7	33.8	33.9
16	27.3	28.3	27.7	27.9
17	50.4	52.9	53.3	53.6
18	12.6	12.4	13.1	13.2
19	19.5	19.4*	19.8*	21.9
20	39.0	33.2	33.7	33.9
21	16.3	19.6*	20.0*	21.4
22	63.0*	40.4	40.9	38.0
23	57.3*	68.8**	69.4**	70.4
24	60.3*	68.2**	68.5**	76.8
25	58.2	59.4	60.2	72.5
26	21.7***	21.2****	21.7	26.3
27	24.5**	24.2***	24.9	27.2
28	24.6**	24.5***	27.3***	27.2
29	21.4***	21.4****	14.8	15.9
30	27.4	27.0	27.9***	27.6

1 and **3** run at 90.56 MHz, **4** at 62.5 MHz.
Spectra run in $CDCl_3$.
* Assignments in the same column with identical asterisks are, interchangeable.

Dihydroniloticin diacetate. **4** (30 mg) was acetylated as described above and the product (**9**) recrystallized from MeOH as needles, mp 162°. Found: M^+ 542.3986; $C_{34}H_{54}O_5$ requires 542.3971. ^1H NMR ($CDCl_3$, 250 MHz): δ 5.25 (1H, *m*, H-7), 4.85 (1H, *ddd*, J = 9, 8, 5 Hz, H-23), 4.52 (1H, *dd*, J = 10.8, 5.7 Hz, H-3), 2.75 (1H, *d*, J = 9 Hz, H-24), 2.09, 2.07 (2 × OAc), 1.37, 1.33 (Me-27, Me-26), 0.97 (3H, *d*, J = 5.7 Hz, Me-21), 0.97, 0.93, 0.85, 0.81, 0.76 (5 × Me).

Oxidation of dihydroniloticin diacetate to the 7,9(11)*-diene* (**10**). The diacetate **9** (45 mg) and mercuric acetate (80 mg) were dissolved in HOAc and allowed to stand for 20 hr. The reaction mixture was filtered, diluted with H_2O, and extracted into $CHCl_3$. The $CHCl_3$ soln was washed with 10% Na_2CO_3 and evapd to give **10** (32 mg) which recrystallized from MeOH as needles, mp 160°. Found: M^+ 540.3831; $C_{34}H_{52}O_5$ requires 540.3815. UV-λ_{max} nm (log ε) 231 (4.05), 237 (4.08), 246 (3.89). ^1H NMR ($CDCl_3$, 250 MHz): δ 5.34, 5.20 (2 × 1 H, 2 × *br s*, H-7 and H-11), 4.85 (1H, *dt*, J = 5.1, 9.0 Hz, H-23), 4.51 (1H, *dd*, J = 10.1, 4.9 Hz, H-3), 2.76 (1H, *d*, J = 9.0 Hz, H-24), 2.07, 2.06 (2 × OAc), 1.37, 1.33 (Me-26, Me-27), 0.99 (3H, *d*, J = 6.0 Hz, Me-21), 0.96, 0.94, 0.87, 0.84, 0.62 (5 × Me). EIMS *m/z* (rel. int.): 540 $[M]^+$ (67), 465 (15), 408 (10), 353 (38), 253 (12), 71 (7), 43 (100). ORD ($CHCl_3$) $[\phi]_{251}$ -4370, $[\phi]_{242}$ −3085.

Piscidinol A **(5)**. Needles from MeOH, mp 198–200° (Lit. [5] 195°); $[\alpha]_D$ −100.6° ($CHCl_3$; *c* 0.2) (Lit. [5] −90°). Found: M^+ 474.3718; $C_{30}H_{50}O_4$ requires 474.3709. ^1H NMR see Table 1. EIMS *m/z* (rel. int.): 474 $[M]^+$ (21), 459 (5), 441 (16), 398 (10), 383 (15), 369 (100), 365 (10), 351 (22), 325 (45), 143 $[C_8H_{15}O_2]^+$ (1), 125 (8), 107 (18).

Hispidol B **(6)**. Needles from MeOH, mp 252° (Lit. [6] 253–254°), $[\alpha]_D$ −46° ($CHCl_3$–EtOH 1:1 ; *c* 0.065) (Lit. [6] −57°). Found: M^+ 476.3864; $C_{30}H_{50}O_4$ requires 476.3865. ^1H NMR see Table 1. EIMS *m/z* (rel. int.): 476 $[M]^+$ (51), 461 (10), 443 (17), 425 (17), 371 (100), 353 (36), 327 (37), 189 (22), 187 (29), 175 (25), 173 (20).

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