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PERFORATINOLONE, A LIMONOID FROM *HARRISONIA PERFORATA*

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Key Word Index—*Harrisonia perforata*; Simaroubaceae; tetranortriterpenoid; limonoid; perforatinolone; perforatin.

Abstract—Perforatinolone, a new tetranortriterpenoid with an A,D-ring *seco*-limonoid structure, which is closely related to perforatin, was isolated from the leaves of *Harrisonia perforata*. Its structure was deduced by spectroscopic data, especially NMR measurements.

INTRODUCTION

Harrisonia perforata (Blanco) Merr. is widely distributed in the mountains of North and Middle Vietnam. It is used in folk medicine for the treatment of itching. In Indonesia and Philippines the root bark is a remedy for diarrhoea, dysentery and cholera [1]. Recently, perforatin (**2**), a novel A,D-*seco*-limonoid, was isolated from this plant [2]. In the family Simaroubaceae, limonoids are known in the species *Harrisonia abyssinica* and *H. perforata* [2, 3]. Very recently, they were also found in the species *H. brownii* [4]. In continuation of our studies on constituents of *H. perforata* we now report the isolation and the structural elucidation of a new limonoid, perforatinolone (**1**). Additionally, gallic acid, sitosterol and 3-*O*-β-D-glucopyranosyl sitosterol were isolated from this plant.

RESULTS AND DISCUSSION

Repeated chromatography on silica gel of the acetone extract of the dried leaves of *Harrisonia perforata* yielded perforatinolone (**1**). Compound **1** gave no molecular ion peak in the EI mass spectrum. It exhibited important peaks at *m/z* 458.1250 $C_{23}H_{22}O_{10}$ $[M - Me_2CO]^+$, 440.1094 $C_{23}H_{20}O_9$ $[M - Me_2CO - H_2O]^+$, 301.1103 $C_{17}H_{17}O_5$, 151.0756 $C_9H_{11}O_2$ and 58 $[Me_2CO]^+$. The FAB mass spectrum afforded an $[M + H]^+$ peak at *m/z* 517, which corresponds to the molecular formula $C_{26}H_{28}O_{11}$.

The ^{13}C NMR spectrum (Table 1) showed, in correspondence with the molecular formula, 26 signals including five methyl groups, which are all quaternary (five singlets in the proton spectrum). In the ^1H NMR spectrum (Table 1) two doublets at δ5.90 (2-H) and 7.02 (1-H) with $J_{1,2} = 9.8$ Hz (δ_C118.8 and 150.9, respectively) belong to a *cis*-disubstituted double bond of an α,β-unsaturated lactone [long range coupling of C-3 (δ161.0) with 1-H and 2-H]. Two other carbonyl carbons appeared at δ168.1 (C-16, lactone) and 202.2 (C-6, ketone). Other functional groups are a hemiketal at δ_C99.9 (C-7) and an epoxy ring [C-15, δ_C57.9, δ_H4.76, long range correlation between 15-H and C-14 (δ70.4)]. All these features showed that **1** is closely related to perforatin (**2**). The carbon and proton shifts of the rings A, B and C of both compounds correspond to each other, so that these rings are identical. In the ring D appeared slight differences suggesting a different structure of the side chain. Thus, instead of the signals of the furan residue in perforatin (**2**), the new compound **1** showed a carbonyl signal (C-21, δ_C170.0), which belonged to an α,β-unsaturated lactone [long range coupling between C-21 and 22-H, (*t*, 7.32, δ_C150.9)]. Another long range coupling between C-21 and the proton at δ6.18 (δ_C97.8) indicated that C-21 is close to a hemiacetal as part of a hydroxy butenolide ring. The complete structure of the new compound **1** was confirmed by the analysis of the CH long range correlations from the HMBC spectrum (Table 1). The striking broadness of the ^{13}C signal of C-23 (half-maximum intensity line width $\Delta\nu_{1/2}$ *ca* 40 Hz) is explained by an equilibrium between the α- and β-hydroxy isomers of the butenolide ring with the open-chained aldehyde form as intermediate. Another strong broadening effect is observed at C-22 and smaller effects at C-20 and C-21. Other limonoids with the same hydroxy butenolide residue such as nimocinolide [5] and nimbocinolide [6] from *Azadirachta indica* and 23-hydroxytoonacilide from *Toona ciliata* [7], show for C-16, C-17, C-20, C-21 and C-22 two distinct signals belonging to the two epimeric forms.

EXPERIMENTAL

Mps are uncorr. MS: 70 eV. FAB-MS: matrix glycerine. NMR spectra: chemical shifts are given in ppm from TMS; HMBC: delay 6.5 msec. TLC: precoated silica

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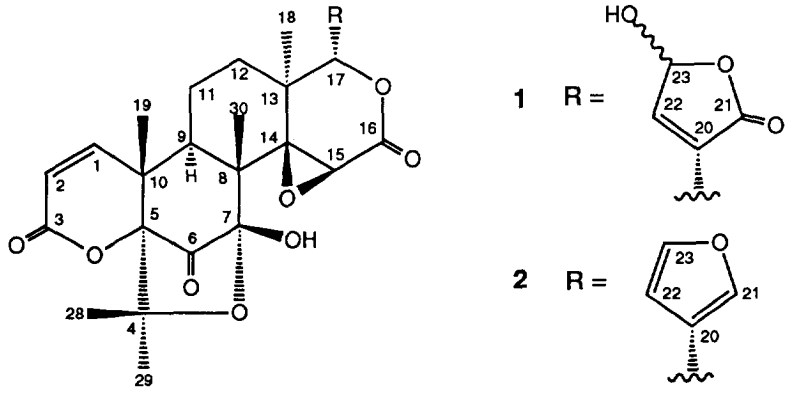

Table 1. NMR spectral data of **1** and **2**

	1*			**2**‖
Pos.	δ_C[ppm]†	δ_H[ppm]‡,§, *J* [Hz]	C–H-long range correlations from HMBC‡	δ_C [ppm]
1	150.9	7.02 *d* (9.8)	9-H, 19-H	152.5
2	118.8	5.90 *d* (9.8)	—	119.0
3	161.0	—	1-H, 2-H	161.1
4	80.2	—	28-H_3, 29-H_3	80.8
5	89.2	—	1-H, 28-H_3, 29-H_3	89.9
6	202.2	—	1-H	202.9
7	99.9	—	30-H_3	101.0
8	51.6	—	9-H, 11-H_2, 30-H_3	52.3
9	35.2	3.10 *dd* (13.1, 6.4)	1-H, 11-H_2, 12-H^A, 30-H_3	36.3
10	44.3	—	1-H, 2-H, 9-H, 19-H_3	45.0
11	15.4	1.83–1.97 *m*	9-H	15.7
12	24.8	A 1.50 *m* B 1.99–2.07*m*	11-H_2, 18-H_3	26.0
13	40.6	—	11-H_2, 12-H_2, 17-H, 18-H_3	40.6
14	70.4	—	9-H, 12-H^A, 15-H, 17-H, 18-H_3, 30-H_3	71.4
15	57.9	4.76 *s*	—	58.8
16	168.1	—	15-H	166.2
17	76.0	5.61 *s*	18-H_3	78.5
18	18.7	1.29 *s*	12-H^B, 17-H	19.9[b]
19	19.5	1.22 *s*	9-H	19.8[b]
20	133.5	—	17-H, 23-H	122.1
21	170.0	—	17-H, 22-H, 23-H	142.6
22	150.9 *br*	7.32 *t* (1.1)	17-H	111.0
23	97.8 *br*¶	6.18 *s*	22-H	144.2
28	27.5[a]	1.29 *s*	29-H	28.1
29	27.8[a]	1.40 *s*	28-H	28.1
30	15.1	1.21 *s*	9-H	15.4

*In $CDCl_3$ with small amounts of CD_3OD.

†100 MHz.

‡125/500 MHz.

§One-bond ^1H–^{13}C correlations were derived from the HMQC.

‖75/300 MHz, in acetone-d_6, from ref. [2]; assignments of C-4 and C-5 were reversed. The assignment of the methyl groups was missing in ref. [2] and was made by comparison with **1**.

¶Half-maximum intensity line width *ca* 40 Hz.

[a,b]Assignment interchangeable.

gel plates 60 F_{254} (Merck), detection: UV-light, spray reagent: vanillin in H_2SO_4.

Plant material. The leaves of *Harrisonia perforata* were collected in the mountains of Son La province, North Vietnam, in September 1991 and identified by Dr T. D. Dai. A voucher specimen is deposited in the Institute of Ecology and Natural Resources, National Centre for Scientific Research and Technology, Hanoi, Vietnam.

Isolation. The dried leaves (700 g) were defatted with *n*-hexane and extracted at room temp. with Me_2CO and EtOH successively and the solvents removed. The residue of the Me_2CO extract (35 g) was extracted with *n*-hexane. Repeated chromatography of the non-soluble part (17 g) over silica gel with increasing amounts of EtOAc in *n*-hexane as eluent afforded gallic acid (30 mg, sublimation at 232–236°, crystallized from H_2O), and perforatinolone (**1**) (17 mg). Mp 245–247°, (crystallized from MeOH). $[\alpha]_{578}^{25}$ −63.4°, $[\alpha]_{546}^{25}$ −82.2° (MeOH; *c* 0.51). IR ν_{max}^{KBr} cm^{-1}: 1794, 1746, 1601 (*br*), 1396, 1260, 1024. MS *m/z* (rel. int.): 458.1250 $C_{23}H_{22}O_{10}$ calc. 458.1287 (7.5), 440.1094 $C_{23}H_{20}O_9$ calc. 440.1108 (1.5), 301.1103 $C_{17}H_{17}O_5$ calc. 301.1130 (7.8), 275.1137 $C_{12}H_{19}O_7$ calc. 275.1142 (2.3), 163.0400 $C_9H_7O_3$ calc. 163.0405 (4.8), 151.0756 $C_9H_{11}O_2$ calc. 151.0759 (25.1). FAB-MS *m/z* (rel. int.): 609 $[M + H + glycerine]^+$ (7), 539 $[M + H + Na]^+$ (9), 517 $[M + H]^+$ (16), 115 (100). The residue of the EtOH extract (52 g) was chromatographed over silica gel. Elution with *n*-hexane–EtOAc (94:6) yielded sitosterol (16 mg, mp. 128–131°, crystallized from Me_2CO). Elution with $CHCl_3$–MeOH (95:5) afforded 3-*O*-β-D-glucopyranosyl-sitosterol (116 mg, mp 285–290°, crystallized from EtOH–$CHCl_3$).

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