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FERN CONSTITUENTS: CYCLOHOPENOL AND CYCLOHOPANEDIOL, NOVEL SKELETAL TRITERPENOIDS FROM RHIZOMES OF *PYRROSIA LINGUA*

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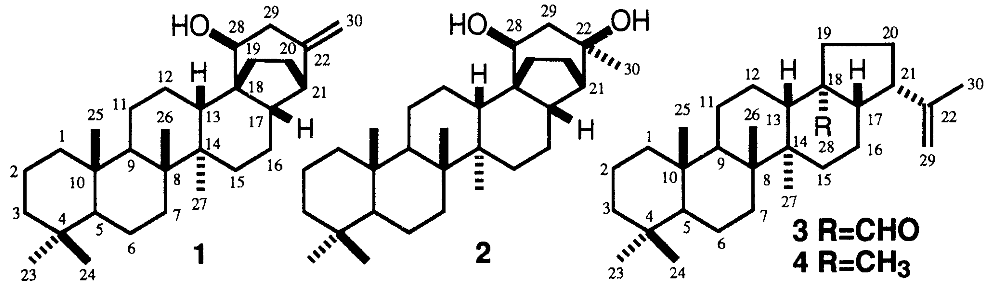
Cyclohopenol (**1**) and cyclohopanediol (**2**), two hexacyclic hopane derivatives, were isolated along with hop-22(29)-en-28-al (**3**) from the rhizomes of *Pyrrosia lingua*. They were characterized as (28*S*)-28,29-cyclohop-22(30)-en-28-ol and (22*R*,28*S*)-28,29-cyclohopane-22,28-diol, respectively, on the basis of spectral analyses.

KEY WORDS triterpenoid; cyclohopenol; cyclohopanediol; fern; *Pyrrosia lingua*; Polypodiaceae

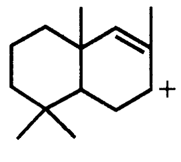
During the course of studies on triterpenoids from polypodiaceous ferns, we reported new oxygenated hopane triterpenoids from the hexane extract of the rhizomes of *Pyrrosia lingua*.[1] Continued investigation on the same extract resulted in the isolation of two novel hexacyclic triterpenoids, designated as cyclohopenol (**1**) and cyclohopanediol (**2**), which were characterized as (28*S*)-28,29-cyclohop-22(30)-en-28-ol and (22*R*,28*S*)-28,29-cyclohopane-22,28-diol, respectively. Another new hopane-type triterpenoid, hop-22(29)-en-28-al (**3**), believed to be the precursor of **1** and **2** in their biogenesis, was also isolated. Compound **1** and **2** are the first reported hopane-type triterpenoids with an additional carbocyclic ring. We report here the structural elucidation of **1**–**3**.

Compounds **1**–**3** were isolated from fresh rhizomes (2.03 kg) collected at Matsuzaki (Shizuoka) in December. Chromatography of the hexane extract over silica gel yielded several fractions which showed positive Liebermann-Burchard test. While the fraction eluted with *n*-hexane-benzene (1 : 1) on further purification through column chromatography followed by preparative HPLC yielded **1** (1.5 mg, 0.0001% of dried plant material) and **3** (13.6 mg, 0.0012%), the fraction eluted with MeOH furnished **2** (5.9 mg, 0.0005%).

The high-resolution mass spectrum of **1** [mp 225–226°C, $[\alpha]_D^{23}$ +43.4° (*c*=0.1, $CHCl_3$); ν_{max}^{KBr} cm^{-1}: 3400, 1160 (OH), 1650, 880 (>C=CH_2), *m/z* (rel. int.) 409 (M^+–CH_3, 5), 406 (M^+–H_2O, 16), 391 (M^+–H_2O–CH_3, 4), 257 (15), 256 (23), 218 (18), 191 (100), 187 (16), 185 (28), 137 (32), 129 (37)] showed a molecular ion at *m/z* 424.3703 (26) corresponding to the molecular formula $C_{30}H_{48}O$. The base peak at *m/z* 191 in its mass spectrum indicated that the compound might belong to the hopane skeleton.[2] Its ^1H-NMR spectrum (Table 1) displayed signals for five tertiary methyl groups, one exo-methylene group, and a secondary hydroxyl group. A comparison of its ^{13}C NMR spectrum with that of hop-22(29)-ene (**4**),[3] the major co-occurring hopane triterpenoid[4] (Table 2) revealed that the two methyl groups of **4** must either be functionalized or involved in some sort of ring formation in **1**. Since **1** contains a hydroxymethine and a methylene carbons at the expense of the two methyl carbons of **4**, it can be presumed that **1** must have a hexacyclic structure. This was corroborated by its molecular formula. Since ^{13}C chemical shifts of C-1 to C-17 with the exception of C-12, and C-23 to C-27 were found to be very close to those of **4**, it was certain that the additional carbocyclic ring must be formed by involving C-29/30 and C-28. However, a detailed analysis of the HMBC spectrum of **1** could not provide conclusive



1 **2** **3** R=CHO
4 R=CH_3



m/z 191 (100)

evidence in favor of this assumption, although the presence of two partial structures shown by heavy lines in **1a** (Fig. 1) could be ascertained. The molecular ion (HR-MS) of **2** [mp 265–266°C, $[\alpha]_D^{23}$ +50.3° (c=0.5, $CHCl_3$); ν_{max}^{KBr} cm^{-1}: 3450, 1120 (OH), m/z (rel. int.): 427 (M^+-CH_3, 2), 424 (M^+-H_2O, 33), 409 ($M^+-H_2O-CH_3$, 7), 406 (13), 232 (9), 219 (11), 191 (100), 174 (34), 137 (14), 123 (11)] at m/z 442.3800 ($C_{30}H_{50}O_2$, 5), which is 18 mass units higher than that of **1**, indicated that **2** might be a hydration product of **1**. Its ^1H and ^{13}C NMR spectra (Tables 1 and 2) showed the presence of six tertiary methyl groups (in place of five in **1**), one hydroxymethine, and one hydroxy quaternary carbon in the molecule. No signals for the exo-methylene group could, however, be observed. Its ^{13}C NMR data (Table 2) clearly revealed a resemblance between the structures of **1** and **2**. The structure of **2** could finally be established based on its HMBC spectrum.

Table 1. ^1H Chemical Shifts[a] (δ ppm, $CDCl_3$, 500 MHz) of **1**–**4**

	H_3-23	H_3-24	H_3-25	H_3-26	H_3-27	H_3-28/H-28	H_2-29	H_3-30/H_2-30	H-13$_\beta$	H-17$_\beta$	H-21
1	0.849	0.790	0.814	0.994	1.111	4.311 (dd, 10.4, 6.7)	1.933 2.367	4.539 (m)	1.53	1.32	2.471 (dd, 5.8, 2.1)
2	0.852	0.791	0.822	0.995	1.131	4.237 (dd, 10.4, 5.5)	1.28 (β) 1.84 (α)	1.338	1.55	1.37	1.84
3	0.835	0.783	0.806	0.994	0.800	10.016	4.495 4.908	1.790	1.69	1.72	2.672 (ddd, 8.7, 8.7, 4.0)

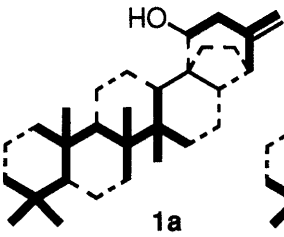
a) Figures in parentheses are coupling constants in Hz. Chemical shifts were assigned on the basis of ^1H-^1H COSY, HSQC, and HMBC spectral analyses.

Table 2. ^{13}C Chemical Shifts (δ, $CDCl_3$, 125 MHz) of **1**–**4**

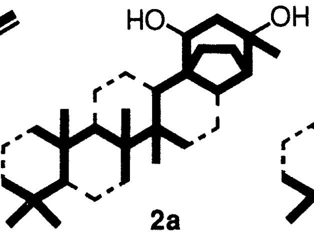
	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10
1	40.29	18.66	42.07	33.25	55.96	18.62	33.57	42.51	50.81	37.32
2	40.28	18.62	42.03	33.22	56.02	18.62	33.66	42.35	51.01	37.32
3	40.24	18.65	42.00	33.23	56.07	18.61	33.90	41.63	50.35	37.38
4	40.31	18.70	42.10	33.25	56.10	18.70	33.25	41.90	50.37	37.39

	C-11	C-12	C-13	C-14	C-15	C-16	C-17	C-18	C-19	C-20
1	21.86	27.20	50.76	42.63	32.52	20.31	53.22	47.91	31.17	29.87
2	22.00	27.20	51.25	42.39	33.40	22.46	51.58	47.11	31.37	26.20
3	20.93	23.94	51.92	42.08	32.76	21.43	53.15	59.63	35.79	27.52
4	20.91	23.99	49.42	42.07	33.61	21.67	54.88	44.80	41.90	27.39

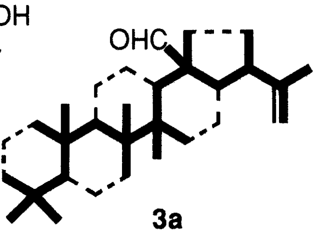
	C-21	C-22	C-23	C-24	C-25	C-26	C-27	C-28	C-29	C-30
1	47.35	148.32	33.39	21.59	15.93	17.00	17.50	71.10	39.61	105.85
2	50.37	72.01	33.32	21.54	16.07	16.81	16.93	69.98	45.73	29.98
3	46.66	146.03	33.35	21.57	15.91	16.61	17.90	208.15	112.60	25.39
4	46.47	148.78	33.41	21.60	15.84	16.70	16.75	16.07	110.06	25.02



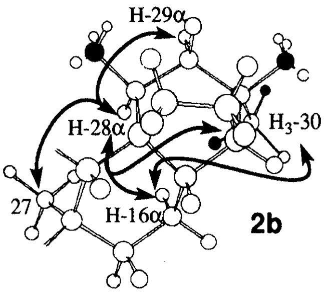
1a



2a



3a



2b

Thus, the two- and three-bond correlations of the methyl protons, the carbinyl proton (H-28), and some methylene protons (H_2-19 and H_2-20) with those of the neighboring carbons clearly showed the presence of the partial structure as in **2a** (Fig. 1). The carbon-carbon connectivities shown by broken lines could be deduced from ^{1}H-^{1}H COSY and HSQC spectral analyses. The stereochemistry at the chiral centers C-22 and C-28 could be established from the NOE interactions, *viz.* H_3-27 $\leftrightarrow$ H-28α, H-28α $\leftrightarrow$ H-29α, H-28α $\leftrightarrow$ H_3-30, H-28α $\leftrightarrow$ H-16α (δ 1.68), and H-16α $\leftrightarrow$ H_3-30, observed in the NOESY spectrum of the compound as depicted in **2b** (Fig. 1). The coupling constants (10.4, 5.5 Hz, Table 1) of the ^{1}H NMR signal for the carbinyl proton (H-28α) of **2** indicated that the new carbocyclic ring F assumes a chair conformation and the hydroxyl group is equatorially oriented. The coupling constants of the H-28 signal of **1** were also very close to those of **2**. Based on the above observations, **1** and **2** may be represented as (28*S*)-28,29-cyclohop-22(30)-en-28-ol and (22*R*,28*S*)-28,29-cyclohopane-22,28-diol, respectively.

The high-resolution mass spectrum of **3** [mp 217–220°C, $[\alpha]_D^{23}$ +145.6° (*c*=0.5, $CHCl_3$); ν_{max}^{KBr} cm^{-1}: 1700 (CHO), 1640, 890 (>C=CH_2), *m/z* (rel. int.): 409 (M^+–CH_3, 13), 395 (M^+–CHO, 7), 381 (M^+–C_3H_7, 3), 353 (14), 312 (4), 271 (4), 233(8), 219 (18), 191 (100), 147 (42), 137 (20)] showed a molecular ion peak at *m/z* 424.3713 (13) corresponding to the molecular formula $C_{30}H_{48}O$. Its ^{1}H-NMR spectrum (Table 1) revealed the presence of six tertiary methyl groups, one exo-methylene group, and one aldehyde proton. The ^{13}C chemical shifts (Table 2) of the compound were found to be very close to those of **4** except for the carbons C-13, C-18, C-19, and C-28. Since C-18 suffered a strong down-field shift by ~15 ppm compared to that of **4**, the aldehyde group of **3** must be located at this carbon. The assigned structure of the compound was finally corroborated by its 2D NMR spectra.

Biogenetically, the cyclohopane triterpenoids **1** and **2** may be formed from **3**, as shown in Chart 1. The double bond at $\Delta^{22(29)}$ may attack the aldehyde carbon forming the cyclohopane cation. Either a proton may be lost from C-30 of the cyclohopane cation to give **1** or a hydroxyl ion may attack the cation center at C-22 to yield **2**.

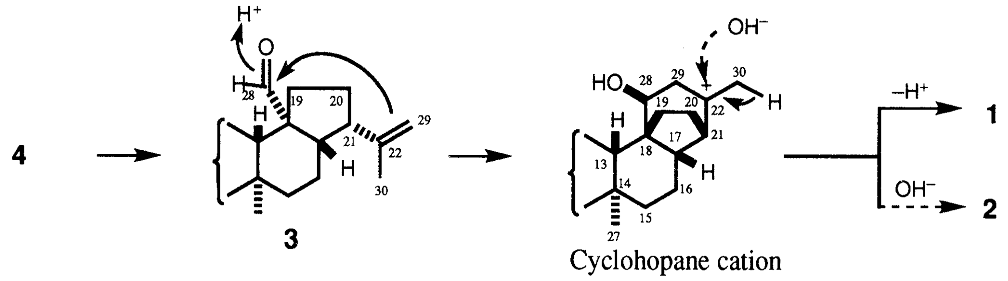


Chart 1

Many hopane-type triterpenoids, for example, oxygenated hopane triterpenoids from this species[1] and bacteriohopane tetrol from *Acetobacter xylinum*,[5] and hopane-29-(2')-pentanone from sediments of various origins[6] with extra carbons at C-29 or C-30, have been reported from natural sources. Cyclohopane triterpenoids **1** and **2** are the first examples with an extra carbon ring in a hopane skeleton.

References and Notes

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