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A new norneohopane caffeate from *Filicium decipiens*

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Abstract

Investigation of the dichloromethane extract of the stem of *Filicium decipiens* yielded a new natural product, 24-norneohopa-4(23),22(29)-diene-3β,6β,7β-triol 7-caffeate **(1)**.

Keywords: *Filicium decipiens*; 24-Norneohopa-4(23),22(29)-diene-3β,6β,7β-triol 7-caffeate; Triterpenoids

1. Introduction

The Sri Lankan flora comprises approximately 13 genera and 24 species of the family Sapindaceae. Out of these, eight species are considered to be endemic to Sri Lanka [1]. In continuation of our studies on Sri Lankan Sapindaceae [2–4] we have chemically investigated *Filicium decipiens* (Wight et Arn.) Thwaites, a tree of moderate size growing in wet and intermediate zones of Sri Lanka [5] and also in southern Africa. Four triterpenoidal saponins have been reported from the stem bark [6]. Preliminary investigations of the dichloromethane, methanol and *n*-butanol

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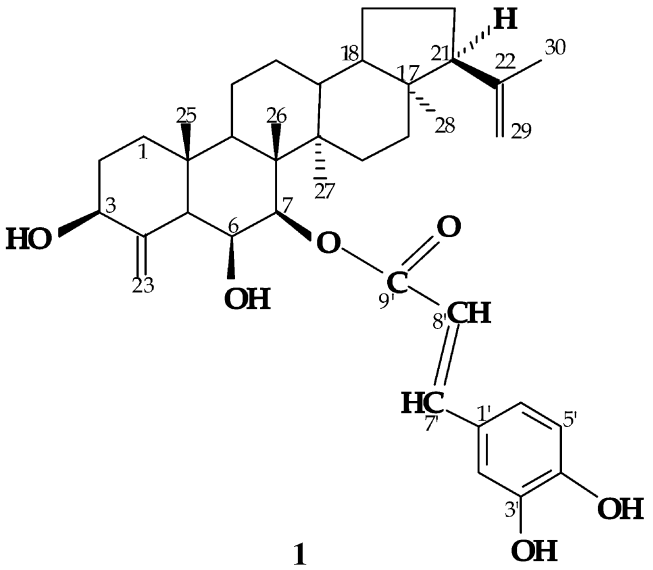
fractions of the methanol extracts from the leaves and the stem showed a variety of biological activities, e.g. antifungal, antibacterial and molluscicidal activities [7].

In this paper we report the isolation and structure elucidation of a new natural product, 24-norneohopa-4(23),22(29)-diene-3β,6β,7β-triol 7-caffeate (**1**) from dichloromethane extract of the stem.



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2. Experimental

2.1. General

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a JEOL EX 400 spectrometer. Positive FAB-MS spectra were obtained on a JEOL JMS-AX505HA spectrometer with glycerol as the matrix.

2.2. Plant material

Mature stems of *F. decipiens* were collected from the Central Province of Sri Lanka, in July 1996 and identified by Mr Aruna Weerasooriya, Royal Botanical Garden, Peradeniya, Sri Lanka. A voucher specimen is deposited at the Institute of Fundamental Studies.

2.3. Extraction and isolation

The dried ground stems (4 kg) were defatted with *n*-hexane and extracted with CH_2Cl_2. Evaporation of the solvent gave a greenish solid residue (27 g). A portion (25 g) was separated by MPLC on a column of silica gel (Merck Art. 9385) followed by gradient elution with *n*-hexane, EtOAc and MeOH. The fraction eluted with 2% MeOH-EtOAc was further purified by Si-gel CC to give compound **1** (60 mg).

(*21α-H*)*-24-norneohopa-4*(*23*),*22*(*29*)*-diene-3*β,*6*β,*7*β*-triol 7-caffeate* (**1**). Colorless microcrystals, mp 278°C; UV max (EtOH): 216, 246, 304, 331 nm; IR bands (KBr): 3450, 2950, 1680, 1670, 1630, 1600, 1520, 1440, 1370, 1270, 1180, 1160, 1110, 1056 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR: see Table 1; FAB-MS(+) *m/z*: 605 $[M + H]^+$, 425 $[M\text{-}C_9H_8O_4 + H]^+$, 407 $[M\text{-}C_9H_8O_4\text{-}H_2O + H]^+$.

3. Results and discussion

The dry ground mature stem and stem bark of *F. decipiens* were defatted with *n*-hexane and extracted with dichloromethane. Chromatographic separation of the dichloromethane extract over silica gel afforded **1** as colorless microcrystals in a yield of 0.0015%. Its molecular weight was suggested by the molecular ion peak at *m/z* 605 $[MH]^+$ in the FAB-MS. The HRFAB-MS gave *m/z* 605.3806 for the $[MH]^+$ ion (calcd. 605.3842), corresponding to the molecular formula $C_{38}H_{52}O_6$. In addition, the FAB-MS showed a prominent ion at *m/z* 425 arising from the loss of a $C_9H_8O_4$ fragment found to be caffeic acid by ^{13}C-NMR analysis of **1** (vide infra). The IR spectrum of **1** showed strong absorptions at 1680 cm^{-1} for a conjugated carbonyl and at 3450 cm^{-1} for hydroxyl(s).

The ^{1}H-NMR spectrum of **1** showed signals of five tertiary methyl groups (one of them being located on an olefinic double bond), three oxymethine protons (one of them being an esterified oxymethine on the basis of the down field chemical shift) and two exomethylene groups, together with signals assignable to a *trans*-caffeoyl (6,7-dihydroxycinnamoyl) moiety. The H-H COSY spectrum revealed the presence of an isopropenyl group and the vicinal relationship for two oxymethine protons. The ^{13}C-NMR spectrum, assisted with DEPT experiments, showed 38 signals (the signal at δ 47.7 being an overlapping of two carbons). The signals at δ 167.2, 147.6, 145.7, 144.8, 126.3, 121.8, 121.8, 114.6 and 113.9 were assigned to the caffeoyl group [8]. In agreement with the ^{1}H-NMR data, carbon signals for three oxymethine carbons (δ 72.3, 71.0 and 75.1) and two exomethylene groups (δ 104.8, 148.8, 109.2 and 147.8) were observed. Absence of any further sp^2 resonances suggested **1** to be a pentacyclic nortriterpene.

Further formulation was done with the aid of HMQC and HMBC spectra recorded in a field-gradient mode. The HMBC correlations are shown in Fig. 1. The exomethylene hydrogens at δ 5.25 and 5.35 were correlated to the oxymethine carbon at δ 72.3 (C-3) and the methine carbon at δ 50.0 (C-5) in addition to the olefinic carbon at δ 148.8 (C-4). The junctional methyl group (25-H_3) was corre-

Table 1
^{1}H and ^{13}C-NMR (400/100 MHz, $CDCl_3$ + CD_3OD) spectra of compound **1**

Position	δ_H	δ_C	HMBC (H → C)
1	1.45, 1.06 (*m*)	40.1	
2	1.93, 1.56 (*m*)	31.4	
3	3.95 (*dd*, *J* 4.8, 11.2 Hz)	72.3	
4	–	148.8	
5	1.76 (*m*)	50.0	C-3, -4, -7, -10, -23, -24
6	4.36 (*dd*, *J* 3.6, 2.0 Hz)	71.0	C-5, -7, -8, -10
7	5.16 (*d*, *J* 3.6 Hz)	75.1	C-8, -14, -26, -9′
8	–	46.2	
9	1.58 (*m*)	48.8	
10	–	38.0	
11	1.30 (*m*)	21.0[a]	
12	1.55, 1.76 (*m*)	20.9[a]	
13	1.56 (*m*)	47.7	
14	–	44.0	
15	1.00, 1.60 (*m*)	34.7	
16	1.52, 1.08 (*m*)	40.1	
17	–	43.8	
18	1.00 (*m*)	53.1	
19	1.52 (*m*)	23.7	
20	1.43, 1.81 (*m*)	26.7	
21	2.20 (*m*)	47.7	C-18, -22, -29
22	–	147.8	
23	5.25, 5.35 (each *s*)	104.8	C-3, -4, -5
25	1.04 (*s*)	15.6	C-1, -5, -9, -10
26	1.56 (*s*)	12.1	C-7, -8, -9, -14
27	1.06 (*s*)	16.9	C-8, -13, -14, -15
28	0.69 (*s*)	14.6	C-16, -17, -18, -21
29	4.64, 4.66 (each *s*)	109.2	C-21, -28, -30
30	1.66 (*s*)	19.6	C-21, -22, -29
Caffeoyl moiety			
1′	–	126.3	
2′	7.09 (*d*, *J* 2 Hz)	113.9	C-3′, -4′, -6′, -7′
3′	–	144.8	
4′	–	147.6	
5′	6.83 (*d*, *J* 8.2 Hz)	115.0	C-1′, -3′
6′	6.98 (*d*, *J* 8.2 Hz)	121.8	C-2′, -4′, -7′
7′	7.59 (*d*, *J* 16.2 Hz)	145.7	C-2′, -6′, -9′
8′	6.29 (*d*, *J* 16.2 Hz)	114.6	C-1′, -9′
9′	–	167.2	

[a] The assignments may be interchangeable.

lated to the above methine carbon along with a methylene carbon (δ 40.1, C-1), a quaternary carbon (δ 38.0, C-10) and another methine carbon (δ 48.8, C-9). These correlations revealed that C-24 carbon is missing and, therefore, one of the exomethylene locates at 4(23) position. The C-3 position was substituted with a

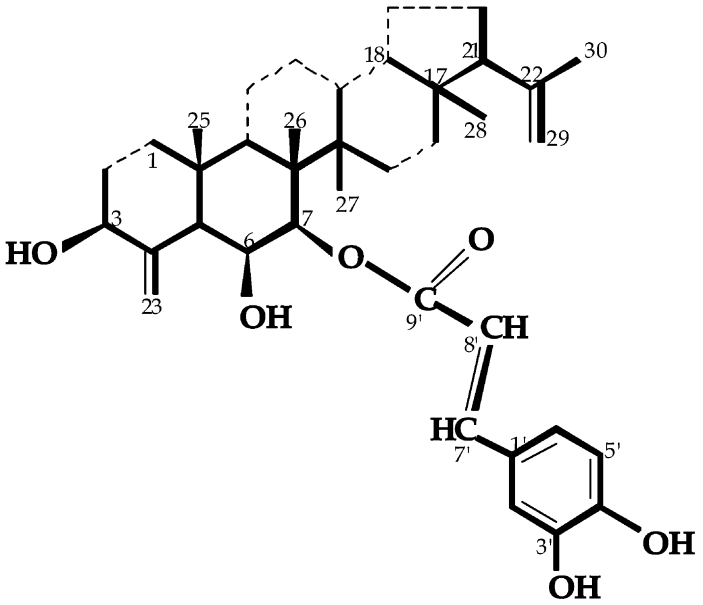

Fig. 1. HMBC correlations of **1** (H → C).

hydroxyl group, and the vicinal oxymethine groups were assigned at the C-6 and C-7 positions. The correlation of the oxymethine proton at δ 5.16 (H-7) to the ester carbonyl at δ 162.7 and of 26-H_3 to the C-7 oxymethine carbon in the HMBC spectrum provided evidence for the presence of 7-caffeate ester. These structural assignments were also supported by the observation of an NOE from H-6 to one (δ 5.35) of the exomethylene protons. At this stage, one may consider 24-norlupane or 24-norhopane skeleton for **1**. However, none of these possibilities was found to fulfill the observed HMBC data. A methane carbon at δ 47.7 (C-21) was correlated to 29-H_2 (δ 4.64/4.66) and 30-H_3 (1.66). Furthermore, the allylic C-21 proton (δ 2.20) was correlated to a junctional methyl at δ 0.69 (28-H_3). These HMBC correlations unambiguously establish the position of the isopropenyl group at C-21 as well as the 24-norneohopane skeleton for compound **1**. The complete ^{1}H and ^{13}C assignments are listed in Table 1.

The stereo-centers of **1** were determined as follows. The hydroxyl group at C-3 was apparently β (equatorial) with a characteristic coupling pattern of 3-H (*dd*, $J = 11.2, 4.8$ Hz) [2]. A NOE was observed between 27-H_3 and 7-H, thus indicating α-orientation (axial) of 7-H and hence β-orientation (equatorial) of 7-*O*-caffeoyl moiety. The C-6 hydroxyl group has β-orientation (axial), since the coupling constants between H-6 and H-7 and between H-6 and H-5 were 3.6 and 2.0 Hz, respectively. The presence of a NOE from H-6 to H-24 (vide supra) would support the stereo-chemical assignment. Furthermore, NOEs were observed between 25-H_3 and 26-H_3, and between 27-H_3 and 28-H_3, supporting the stereo-chemistry for the ring junctions usually encountered in neohopanes. Finally, in the NOE experiments

irradiating 28-H_3, signal enhancements were observed for 27-H_3 as well as for H-21, thus establishing the β-orientation of the isopropenyl group. Hence, the structure of compound **1** was defined as (21α-H)-24-norneohopa-4(23),22(29)-diene-3β,6β,7β-triol 7-caffeate. This is the first report of both a 21α-H-neohopane and a 24-norneohopane. Furthermore, this is the first isolation of a triterpene caffeate from Sapindaceae family, although esters of this kind have been reported from several plants such as *Quercus* (Fagaceae), *Betula* (Betulaceae), *Myrica* (Myricaceae), *Pyracantha* (Rosaceae), *Larrea* (Zygophyllaceae), *Rhoiptelea* (Rhoipteleaceae) and *Zizyphus* (Rhamnaceae) species [9].

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