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FAGAROPSINE, A DEGRADED LIMONOID GLUCOSIDE FROM *FAGAROPSIS GLABRA*

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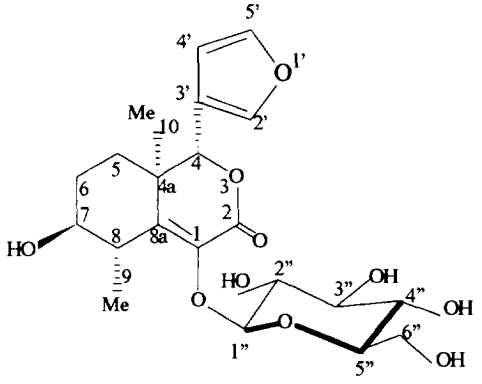
Key Word Index—*Fagaropsis glabra*; Rutaceae; limonoid; degraded limonoid glycoside; fagaropsine.

Abstract—Phytochemical studies of the alcoholic-soluble portion of *Fagaropsis glabra* have resulted in the isolation of fagaropsine, a degraded limonoid glycoside. Its absolute structure was elucidated as 1-*O*-β-D-glucopyranosyl-4α-(3′-furanyl)-7β-hydroxy-4aα,8α-dimethyl-4,4a,5,6,7,8-hexahydro-3-benzopyran-2-one on the basis of spectral data.

INTRODUCTION

Previous investigations of *Fagaropsis* species related the presence in this genus of benzophenanthridine alkaloids [1, 2] and limonoids of the limonidic tetranortriterpenoid [1, 3] and degraded limonoid class [3–5]. The various biological activities of these limonoids are of agricultural and medicinal interest [6]. In the field of antifeedants research, structure–activity relationships [7] led chemists to focus on the C/D ring of these compounds [8, 9] which are naturally encountered as degraded limonoids. In addition to previous degraded limonoids found in *F. glabra* [3, 5], we report here the isolation and identification of the first glucosylated degraded limonoid glucoside (**1**) called fagaropsine, which differs in the D-ring glycosylation pattern from other reported β-D-limonoid glycosides [10].



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RESULTS AND DISCUSSION

As the electronic impact mass spectrum of **1** did not afford any positive result, the molecular weight was determined by FAB mass spectrometry. The positive ion FAB mass spectrum showed the pseudomolecular ion peak $[M+H]^+$ at *m/z* 441 corresponding to $C_{21}H_{28}O_{10}$ and the base peak at *m/z* 279 indicated the presence of a hexose $[(M+H)-(180+H_2O)]^+$. The IR spectrum of **1** exhibited a carbonyl absorption at 1712 cm^{-1} suggesting a pyrano-type aglycone with a conjugated δ-lactonic ring.

These results were corroborated by an extensive analysis of the NMR data (Tables 1 and 2). The comparison with signals observed for degraded limonoids we previously isolated [3, 5], in addition to signals corresponding to a sugar moiety, indicated a glycosylated compound of the pyroangolenside type [8, 11]. The coupling constant $J = 6.5$ Hz of the anomeric proton resonating at δ4.78 and the ^{13}C and ^1H NMR signals were consistent with a β-D-glucose substitution. The molecular mass indicated a further oxygen substituent. Homodecoupling experiments and a 2D COSY-45 homonuclear spectrum clarified most of the ambiguous ^1H coupling systems and confirmed the presence of a H-7 carbinylic signal located at δ3.86 and partially overlapping with H-6A of the glucose. The broad singlet shape of H-7 suggested its equatorial position which was confirmed by ROEs observed between H-7, and the two H-6 and Me-8. In addition, the absence of ROEs between H-7 and the sugar protons suggested a C-1 *O*-glucosidic substitution. Conclusive evidence was given by the HMBC spectrum where a $^3J_{H-C}$ cross-peak appeared between C-1 (δ139.3) of the aglycone and H-1 (δ4.78) of the sugar. Moreover, the

Table 1. ^{1}H NMR (300 MHz) data of fagaropsine in CD_3OD

H	δ J (Hz)
Aglycone	
4	5.26 *s*
5_{ax}	1.85 *td* (12.5, 6.0)
5_{eq}	1.13*
6_{ax}	1.95 *tt* (12.5, 2.5)
6_{eq}	1.68 *br dd* (12.5, 6.0)
7	3.84 *br s**
8	3.53 *qt* (7.6, 1.5)
Me-4a	1.16 *s*
Me-8	1.20 *d* (7.6)
2′	7.59 *dd* (1.7, 0.8)
4′	6.50 *dd* (2.0, 0.8)
5′	7.53 *dd* (2.0, 1.7)
β-D-glucose	
1″	4.78 *d* (6.5)
2″	3.41*
3″	3.39*
4″	3.38*
5″	3.34*
6″A	3.84* *dd* (12.0, 2.1)
6″B	3.68 *dd* (12.0, 5.0)

*Overlapped signals.

Table 2. ^{13}C NMR (75 MHz) data of fagaropsine in CD_3OD

C	δ	Gated Dec. J_{C-H} (Hz)
Aglycone		
1	139.3 *s*	
2	165.2 *s*	
4	82.6 *d*	(149.5)
4a	39.6 *s*	
5	28.9 *t*	(129.5)
6	23.5 *t*	(130.0)
7	71.2 *d*	(145.3)
8	37.0 *d*	(133.3)
8a	155.0 *s*	
9	19.7 *q*	(129.5)
10	19.5 *q*	(129.5)
2′	142.9 *dd*	(199.0, 10.5)
3′	121.2 *br s*	
4′	111.2 *dd*	(174.0, 14.0)
5′	144.5 *ddd*	(204.2, 10.0, 9.0)
β-D-glucose		
1″	105.2 *d*	(134.4)
2″	75.4 *d*	(145.7)
3″	77.8 *d*	(141.9)
4″	71.1 *d*	(145.3)
5″	78.5 *d*	(141.3)
6″	62.5 *t*	(142.2)

assignment of the ^{1}H and ^{13}C NMR resonances of **1** was supported by the HMBC spectrum and the sequence of the sugar protons which appeared as unresolved signals at δ3.30–3.45 in the 1D ^{1}H NMR, can be determined as H-2″, 3″, 4″, 5″ (Table 1).

The high field position of the angular methyl group signal in the ^{1}H NMR spectrum indicated its position *cis* to the furan ring ($\simeq$ +0.4 ppm in *trans*-isomers) [8] and supported the normal limonoid stereochemistry. The comparison with NMR spectra of previously identified degraded limonoids [3–5, 11] and particularly dictamdiol, an aglycone isomer [3, 12], suggested that ring C had a chair conformation. A W-like coupling (1.5 Hz) was present between H-6_{eq} (δ1.68) and H-8 (δ3.53) which inferred the axial (α)-position of the 8-methyl group. Thus, the structure of fagaropsine (**1**) was deduced to be 1-*O*-β-D-glucopyranosyl-(4*R*,4a*R*,7*S*,8*S*)-4-(3′-furanyl)-7-hydroxy-4a,8-dimethyl-4,4a,5,6,7,8-hexahydro-2*H*-3-benzopyran-2-one as the nomenclatural system adopted refers to its limonoid structure.

This compound is the first natural degraded limonoid isolated in a glucosylated form. It suggests the presence in this species of another biosynthetic route in limonoid metabolic glycosylation in contrast to the numerous 17-*O*-β-glucopyranosyl limonoids found in *Citrus* [10, 13] or *Tetradium* [14] species.

EXPERIMENTAL

General. Mps: uncorr. Analyt. TLC was on silica gel GF_{254} and furanyl compounds were visualized with Ehrlich reagent spray followed by immersing in HCl vapour. ^{1}H and ^{13}C NMR spectra were obtained in CD_3OD at 300.13 and 75.45 MHz, respectively, using TMS as int. standard. FAB-MS were recorded in glycerol and glycerol + KI matrix.

Plant material. Fagaropsis glabra Capuron trunk bark was collected in the Sambava country (NE of Malagasy Republic) and authenticated at source by the ORSTOM centre of Tananarive where a voucher specimen is deposited with the reference number : 59.

Extraction and isolation. Dried powdered trunk bark (900 g) of *F. glabra* was defatted with petrol and then successively extracted with CH_2Cl_2 (12 l) and EtOH 90° (15 l). The ethanolic extract was chromatographed over Amberlite XAD-4. The extract eluted with MeOH–H_2O (3 : 1) contained compounds reacting with Ehrlich reagent and was flash-chromatographed over silica gel. Crude fagaropsine was eluted with EtOAc–MeOH (9 : 1) and purified over a Bond Elut C_{18} eluted with MeOH–H_2O (3 : 2) to afford 12 mg of **1**.

Fagaropsine **1**. Mp 165–170°; UV λ_{max}^{MeOH} nm (log ε): 204 (3.8), 238sh; FT-IR ν_{max}^{KBr} cm^{-1} : 3430 (hydroxy), 1712, 1650 (conjugated δ-lactone), 1504, 1070, 1026, 928, 876, 764, 728 (3-substituted furan); FAB-MS *m/z* (rel. int.): 441 ([M + H]$^+$; $C_{21}H_{28}O_{10}$) (100), 279 [(M + H) − (glucose + H_2O-]$^+$ (90); ^{1}H and ^{13}C NMR: Tables 1 and 2.

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