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# QUINOVIC ACID GLYCOSIDES FROM *NAUCLEA DIDERRICHII*

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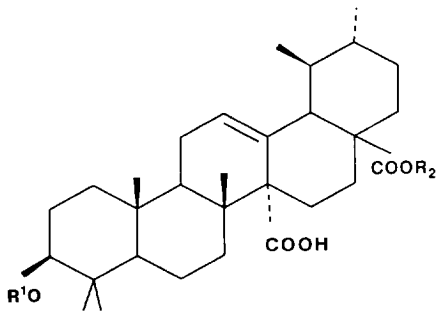
**Key Word Index**—*Nauclea diderrichii*; Rubiaceae; triterpenoid saponins; quinovic acid.

**Abstract**—Three saponins were isolated from stems of *Nauclea diderrichii* and their structures established. These saponins are described for the first time in this plant. Two of them are new compounds.

## INTRODUCTION

*Nauclea diderrichii* (de Wild) Merr is a traditional medicinal plant extensively used in West and Central Africa. The decoctions from the barks and the leaves are used for the treatment of stomach pains, fever and diarrhoea. [1–3]. The isolation of alkaloids and secoiridoids from this plant has been reported [4–7], but to date knowledge about the triterpenoid constituents is scanty. From the bark only quinovic, oxoquinovic acid and the quinovic acid 3-*O*-glucoside have been isolated [7].

In this paper, we describe the isolation and structure elucidation of three saponins from *N. diderrichii*. Saponin **1** has been previously isolated from *Uncaria tomentosa* [8], but is reported now for the first time in *N. diderrichii*. Two of the compounds are new; saponin **2** is quinovic acid-3-*O*-α-L-rhamnosyl (28→1)β-D-glucopyranosyl ester and saponin **3** is quinovic acid-3-*O*[β-D-glucopyranosyl(1→2)β-D-glucopyranoside].




| | $R_1$ | $R_2$ |
|---|---|---|
| 1 | Fuc | Glu |
| 2 | Rha | Glu |
| 3 | Glu 2 -- 1 Glu | H |

Fuc = β - D fucopyranose

Rha = α - L rhamnopyranose

Glu = β - D glucopyranose

## RESULTS AND DISCUSSION

The saponins were extracted from the dried and powdered barks with a mixture of water and methanol (2:8 V/V) and isolated by preparative liquid chromatography on a silica column eluted with mixtures of $CHCl_3$–methanol–water. The complete structures were determined by acid and basic hydrolysis and by $^1H$ and $^{13}C$ NMR spectroscopy and FAB-mass spectrometry (see Experimental).

Acid hydrolysis of **1** yielded fucose and glucose (TLC). Alkaline hydrolysis provided a monodesmoside. The FAB-mass spectrum gave a peak at *m/z* 793 $[M-H]^-$ corresponding to the molecular formula $C_{42}H_{66}O_{14}$ which was confirmed by $^{13}C$ NMR and DEPT spectral data. In the $^{13}C$ NMR spectrum ($CD_3OD$) [Table 1] signals at δ 177.9 and 90.6 indicated that positions C-27 or C-28 and C-3 of the genin were not free. Thus, **1** was a bidesmoside. The genin was identified as quinovic acid by comparison with its literature data [9].

The $^{13}C$ NMR spectrum exhibited signals ascribable to a β-D-fucopyranose linked at C-3 of the genin [9].

The presence in glycoside **1** of a glucose unit linked at the C-28 carboxyl group of the genin was determined

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Table 1. $^{13}C$ NMR data of **1**–**3** ($CD_3OD$)

| Aglycone carbon | 1 | 2 | 3 | DEPT | Sugar carbon | 1 | 2 | 3 |
|---|---|---|---|---|---|---|---|---|
| 1 | 40.0 | 40.0 | 40.0 | $CH_2$ | | Fucose | Rhamnose | Glucose |
| 2 | 27.1 | 26.7 | 26.5 | $CH_2$ | | at C-3 | at C-3 | at C-3 |
| 3 | 90.6 | 90.4 | 91.4 | CH | 1′ | 107.1 | 104.4 | 104.5 |
| 4 | 40.3 | 40.3 | 40.4 | C | 2′ | 73.1 | 72.5 | 81.1 |
| 5 | 57.0 | 56.7 | 56.9 | CH | 3′ | 75.3 | 72.5 | 78.5 |
| 6 | 19.3 | 19.4 | 19.3 | $CH_2$ | 4′ | 72.9 | 74.0 | 71.9 |
| 7 | 37.8 | 37.9 | 37.7 | $CH_2$ | 5′ | 71.6 | 69.9 | 77.7 |
| 8 | 40.8 | 40.8 | 40.8 | C | 6′ | 17.1 | 17.8 | 63.1 |
| 9 | 48.1 | 48.1 | 48.0 | CH | | | | |
| 10 | 38.0 | 38.0 | 38.1 | C | | | | Glucose |
| 11 | 23.9 | 23.9 | 23.9 | $CH_2$ | | | | at C-3 |
| 12 | 130.9 | 130.9 | 130.3 | CH | 1″ | | | 105.4 |
| 13 | 133.8 | 133.4 | 133.5 | C | 2″ | | | 76.3 |
| 14 | 57.3 | 57.4 | 57.3 | C | 3″ | | | 78.4 |
| 15 | 26.4 | 26.5 | 26.4 | $CH_2$ | 4″ | | | 71.6 |
| 16 | 25.8 | 25.8 | 25.8 | $CH_2$ | 5″ | | | 77.9 |
| 17 | 49.8 | 49.8 | 49.5 | C | 6″ | | | 62.9 |
| 18 | 55.3 | 55.4 | 55.6 | CH | | | | |
| 19 | 40.1 | 39.8 | 40.0 | CH | | | | |
| 20 | 38.3 | 38.3 | 38.4 | CH | | Glucose | at C-28 | |
| 21 | 31.2 | 31.2 | 31.3 | $CH_2$ | 1‴ | 95.6 | 95.6 | |
| 22 | 37.0 | 37.0 | 37.0 | $CH_2$ | 2‴ | 73.9 | 74.1 | |
| 23 | 19.2 | 19.2 | 19.1 | Me | 3‴ | 78.7 | 78.6 | |
| 24 | 28.5 | 28.8 | 28.5 | Me | 4‴ | 71.2 | 71.3 | |
| 25 | 16.9 | 16.9 | 16.9 | Me | 5‴ | 78.6 | 78.3 | |
| 26 | 18.1 | 18.2 | 18.2 | Me | 6‴ | 62.5 | 62.6 | |
| 27 | 179.1 | 179.2 | 179.2 | C | | | | |
| 28 | 177.9 | 178.0 | 182.0 | C | | | | |
| 29 | 17.1 | 17.0 | 17.0 | Me | | | | |
| 30 | 21.5 | 21.5 | 21.5 | Me | | | | |

using HMQC and HMBC [10]. The HMQC sequence [$^1H$ detected one-bond heteronuclear multiple quantum coherence] established the connectivity between C-18 ($\delta$55.3) and H-18 ($\delta$2.3; *d*, $J = 10.5$ Hz). In the HMBC [heteronuclear multiple quantum bond connectivity] spectrum long range connectivity ($^3J$) was observed between H-18 and the carboxylic resonance of the genin C-28 ($\delta$177.9). This later resonance also presented a correlation peak with the anomeric proton of the glucose H-1. Therefore, these results indicated esterification of the carboxyl group (C-28) with a $\beta$-D-glucose moiety. Consequently, the structure of **1** was that of quinovic acid 3-*O*-$\beta$-D-fucopyranosyl-(28→1)-$\beta$-D-glucopyranosyl ester.

Saponin **2** provided a monodesmoside on alkaline hydrolysis. Acid hydrolysis of saponin **2** yielded rhamnose and glucose (TLC). The FAB-mass spectrum of **2**, in negative ion mode, showed a quasi-molecular anion at *m/z* 793 and a major fragment at *m/z* 587 suggesting the loss of a carboxyl group and a glucose unit. The molecular formula, $C_{42}H_{66}O_{14}$, was confirmed by the NMR data. All carbon signals of the genin (Table 1) could be assigned by comparison with the $^{13}C$ NMR spectra of the related quinovic acid glycosides [11–13] and those of **1**. The $^{13}C$ NMR spectrum ($CD_3OD$) of **2** (Table 1) was characteristic of a bidesmoside with C-3 at $\delta$90.4 and C-28 at $\delta$178.0. The assignment of the carbon atoms of an esterified glucose was carried out by comparison with the spectrum of **1**.

The presence in **2** of $\alpha$-L-rhamnopyranose linked at the C-3 of quinovic acid was deduced by comparison with previously reported $^{13}C$ NMR data [10, 11]. From these the structure, quinovic acid-3-*O*-$\alpha$-L-rhamnopyranosyl(28 → 1)-$\beta$-D-glucopyranosyl ester, was assigned to **2**.

Alkaline hydrolysis showed that **3** was a monodesmoside. Acid hydrolysis yielded glucose only (TLC).

The FAB-mass spectrum gave a peak at *m/z* 809 $[M-H]^-$ corresponding to the molecular formula $C_{42}H_{66}O_{15}$. The genin was identified as quinovic acid by comparison of $^1H$ and $^{13}C$ NMR data with those of **1** and **2**. The signal at $\delta$182.0 due to C-28 of the genin indicated that the carboxyl group was free.

The downfield shift of the signal for C-3 ($\delta$91.4) showed that a sugar chain was located at this position. The presence of the disaccharide, $\beta$-D-glucopyranosyl(1→2)-$\beta$-D-glucopyranoside linked at the C-3 of the genin was deduced by comparison with previously reported data [12]. The structure of **3** was elucidated as quinovic acid 3-*O*-$\beta$-D-glucopyranosyl(1→2)-$\beta$-D-glucopyranoside.

Saponins **1**–**3** are described here for the first time in *N. diderrichii*. Saponin **1** is a known compound, previously

isolated from *Uncaria tomentosa* [8]. Saponins **2** and **3** are new compounds.

## EXPERIMENTAL

*General experimental procedure.* FAB-MS was in a 10-10H Nermag mass spectrometer in the negative ion mode. All NMR spectra were recorded on a Bruker AMX 400 spectrometer in $CD_3OD$ solns; TMS was used as standard in $^1H$ and $^{13}C$ measurements.

$^{13}C$ Resonance multiplicities were established via the acquisition of DEPT spectra [14]. Standard Bruker pulse sequences were used for both direct and long-range heteronuclear correlation experiments. For other experimental details see Faure *et al.* [15].

*Extraction and isolation.* Plant material was collected in the vicinity of Libreville (Gabon) in August 1992. A voucher specimen is kept in the Department of Pharmacognosy, Faculty of Pharmacy, Marseille (France). Dried and powdered barks (500 g) were treated with $H_2O$–MeOH (2:8). After concn, the $H_2O$ layer was freeze-dried. The residue (20 g) was solubilized in MeOH and subjected to chromatography on a column of activated charcoal (activated carbon, J.T. Baker). The methanolic extract was evapd to dryness (12 g). The residue was partitioned on polyamide (polyamide polycaprolactam Macherey Nagel SC6) with a gradient of MeOH in $H_2O$. After evapn the fr. $H_2O$–MeOH (6:4) gave a residue (1 g) which was submitted to prep. HPLC on a silica gel column (silica gel 230–400 Mesh Merck), with $CHCl_3$–MeOH–$H_2O$ (80:20:2). Saponin **1** was obtained (500 mg). The fr. $H_2O$–MeOH (4:6) (1.2 g) was subjected to chromatography on silica gel and eluted with a mixt. $CHCl_3$–MeOH–$H_2O$ (80:20:2) to yield **2** (150 mg) and **3** (22 mg).

*TLC.* TLC analyses of sugars and saponins were performed on precoated silica gel plates (Kieselgel 60F 254, 0.25 mm; Merck) using the following solvent systems: EtOAc–$HCO_2H$–HOAc–$H_2O$ (100:11:11:27) [system 1]; $CHCl_3$–MeOH–$H_2O$ (80:20:2) [system 2]; $CHCl_3$–$Me_2CO$ (50:6) [system 3]; $CH_2Cl_2$–MeOH–$H_2O$ (50:25:5) [system 4]. Plates were developed with phosphoric acid naphtoresorcinol for sugars, and $H_2SO_4$ for glycosides and genins followed by heating at 110°.

*Alkaline hydrolysis.* The saponin (2 mg) in 0.2% aq. KOH (2 ml) was heated at 100° in a sealed tube for 75 min. After acidification with HCl (pH 5), the monodesmoside was extracted *n*-BuOH; TLC analysis was performed using $CHCl_3$–MeOH–$H_2O$ (80:20:2) [system 2].

*Acid hydrolysis.* The saponin (2 mg) was heated with aq. 10% HCl (2 ml) in a sealed tube at 100° for 4 hr. The sapogenin was extracted with $Et_2O$; then the aq. layer was neutralized with N,N-dioctylamine (10% in $CHCl_3$) and dried. The sapogenin and sugars were identified by TLC analysis with authentic samples in systems 3 and 4, respectively.

*Quinovic acid*-3-O-β-D-*fucopyranosyl*-(28→1)-β-D *glucopyranosyl ester* (**1**). Amorphous powder; TLC (system 1) $R_f$ 0.73; (system 2) $R_f$ 0.32. FAB-MS *m/z*: 793 $[M-H]^-$, 587 $[(M-H)-162-CO_2]^-$, 441 $[(M-H)-162-146-CO_2]^-$. $^{13}C$ NMR ($CD_3OD$): Table 1; $^1H$ NMR ($CD_3OD$): Table 2.

Table 2. $^1H$ NMR data of **1** in $\delta(CD_3OD)$*

| Aglycone proton | **1** |
|---|---|
| Me-23 (3H, *s*) | 0.82 |
| Me-26 (3H, *s*) | 0.87 |
| Me-29 and 30 (6H, *d*) | 0.91 |
| Me-25 (3H, *s*) | 0.97 |
| Me-24 (3H, *s*) | 1.02 |
| H18 (*d*, $J=10.5$ Hz) | 2.30 |
| **Sugar proton*** | |
| Fuc-Me (3H, *d*, $J=6$ Hz) | 1.25 |
| H′1 (1H, *d*, $J=7$ Hz) | 4.22 |
| H″1 (1H, *d*, $J=8.1$ Hz) | 5.40 |

*Determined from HMQC measurement.

*Quinovic acid*-3-O-α-L-*rhamnosyl*-(28→1)-β-D-*glucopyranosyl ester* (**2**). Amorphous powder; TLC (system 1) $R_f$ 0.83; (system 2) $R_f$ 0.3. FAB-MS *m/z*: 793 $[M-H]^-$, 587 $[(M-H)-162-CO_2]^-$, 441 $[(M-H)-162-146-CO_2]^-$. $^{13}C$ NMR ($CD_3OD$): Table 1.

*Quinovic acid*-3-O-β-D-*glucopyranosyl*-(1→2)-β-D *glucopyranoside* (**3**). Amorphous powder; TLC (system 1) $R_f$ 0.50; (system 2) $R_f$ 0.10. FAB-MS *m/z*: 809 $[M-H]^-$, 647 $[(M-H)-162]^-$, 603 $[(M-H)-162-CO_2]^-$, 441 $[(M-H)-162-162-CO_2]^-$. $^{13}C$ NMR ($CD_3OD$): Table 1.

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