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Constituents of *Nelumbo nucifera* leaves and their antimalarial and antifungal activity

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Abstract

From the leaves of *Nelumbo nucifera* (an aquatic plant), one new compound, 24(*R*)-ethylcholest-6-ene-5α-ol-3-*O*-β-D-glucopyranoside (**1**), along with 11 known metabolites (**2**–**12**), were isolated and identified by spectroscopic methods including 1D- and 2D NMR. Antifungal activity for (*R*)-roemerine (**3**) (IC_{50}/MIC = 4.5/10 μg/mL against *Candida albicans*) and antimalarial activity for (*R*)-roemerine (**3**) and *N*-methylasimilobine (**5**) (IC_{50} = 0.2 and 4.8 μg/mL for the D6 clone, respectively, and 0.4 and 4.8 μg/mL for the W2 clone, respectively) was observed. None of the compounds were cytotoxic to Vero cells up to a concentration of 23.8 μg/mL. NMR data for 10-eicosanol (**7**) and 7,11,15-trimethyl-2-hexadecanone (**10**) are presented for the first time. An analysis of the structure–activity relationship shows that the substituents in position C-1 and C-2 of aporphine alkaloids are crucial for the antimalarial activity.

Keywords: *Nelumbo nucifera*; Nelumbonaceae; 24(*R*)-Ethylcholest-6-ene-5α-ol-3-*O*-β-D-glucopyranoside; Roemerine; Antimalarial; SAR

1. Introduction

Nelumbo nucifera Gaertn. (Nelumbonaceae), commonly known as lotus, is a perennial aquatic plant grown and consumed throughout Asia. All parts of *N. nucifera* have been used for various medicinal purposes in oriental medicine. Lotus is reported to possess antidiarrheal, psychopharmacological, diuretic, antipyretic, antimicrobial and hypoglycemic activities (Rai, Wahile, Mukherjee, Saha, & Mukherjee, 2006). Previous work on the leaves of this plant resulted in the isolation of several alkaloids and other constituents (Kashiwada et al., 2005; Wassel, Saeed, Ibrahim, & El-Eraqy, 1996). As part of our on going search for antimicrobial and antimalarial compounds from higher plants, we have undertaken an investigation of the leaves of this plant. In this study, we describe the isolation, structure elucidation and biological activities of a new (**1**) and 11 (**2**–**12**) known compounds (Fig. 1) from the leaves of *N. nucifera* and some structure activity relationship (SAR) for the antimalarial activity of aporphine alkaloids.

2. Results and discussion

Compound **1** was isolated as a white solid. Its molecular formula of $C_{35}H_{60}O_7$, was determined by HRESIMS and indicated the presence of six degrees of unsaturation. ^{13}C NMR and DEPT spectra showed 35 signals including 6 methyls, 11 methylenes, 15 methines and 3 quaternary carbons. Careful examination of the ^{1}H-, ^{13}C NMR and their 2D long-range correlations (Fig. 2) and comparison of aglycone values with literature indicated that compound **1** was a glycoside of the previously reported aglycone 24-ethyl-cholest-6-ene-3β,5α-diol (Greca, Fiorentino, Molinaro, Monaco, & Previtera, 1994). Cross-peak correlations for H-4 (δ 3.18) to C-2 (δ 30.3), C-3 (75.6) and with C-5 (δ 83.1) in the HMBC spectrum was used to place a further hydroxyl at the C-5 position and double bond protons present at δ 5.71 and 5.95 also showed HMBC correlations with C-5 (δ 83.1) indicating that there was a double

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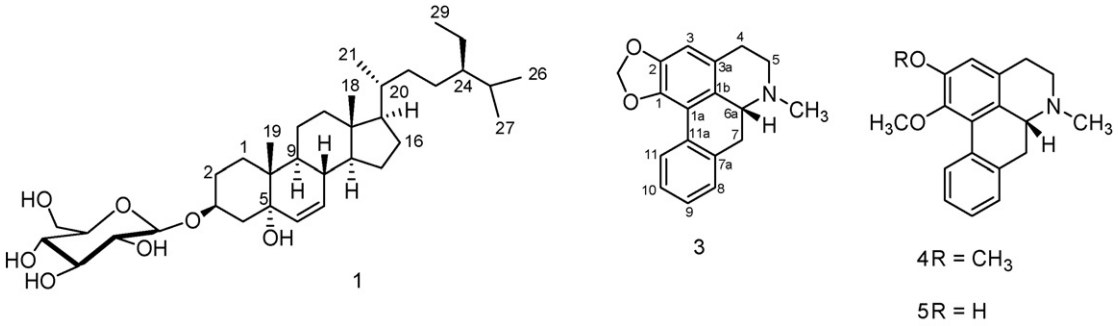

Fig. 1. Structures of some constituents of *Nelumbo nucifera*.

bond between C-6 and C-7. The hydroxyl group at C-5 was determined to be α-oriented (Holland & Jahangir, 1983) from the signals observed in the ^{13}C NMR for C-6 (δ = 133.9) and C-7 (δ = 132.2). The absolute configuration at C-24 was determined to be *R* (Wright et al., 1978) on the basis of the comparison of ^{13}C NMR of **1** (δ_C = 46.4) and β-sitosterol-3-*O*-β-D-glucopyranoside (**11**) (δ_C = 46.4, having an *R* configuration at C-24) in pyridine-d_5. The configuration of the anomeric carbon was defined as β from the coupling constant of 8.0 Hz. *In situ* acid hydrolysis of **1** afforded D-glucose. According to the molecular rotation formula (Klyne, 1950), the specific rotation of **1** ($[\alpha]_D^{26}$: −14°) was multiplied by its molecular wt (*m*/*z* 592), the resulting value (−8288) was then divided by 100. The molecular rotation $[M]_D^{\alpha}$ was found to be −82.9° and is comparable with levorotatory Me-β-D-glucopyranoside ($[M]_D^{\alpha}$ = −66°) (Germonprez, Puyvelde, Maes, Tri, & Kimpe, 2004). According to the molecular rotation calculation, the glucose in **1** should possess the absolute configuration D-form, which is the common form for glucose existing in nature. The glycosidation position was unambiguously determined by a three-bond correlation between the glycosyl anomeric proton H-1′ (δ_H = 5.13) and C-3 (δ_C = 75.6) using HMBC. On the basis of the above evidence, the structure of **1** was established as 24(*R*)-ethyl-cholest-6-ene-5α-ol-3-*O*-β-D-glucopyranoside, a new steroid glucoside.

Eleven known compounds were identified as, dehydroroemerine (**2**), (*R*)-roemerine (**3**), nuciferine (**4**), *N*-methylasimilobine (**5**), and anonaine (**6**) (Guinaudeau, Leboeuf, & Cave, 1975, 1983), 10-eicosanol (**7**), 3,7,11,15-tetramethyl-1-hexadecen-3-ol (isophytol) (**8**) (Ahmad & Ali, 1991), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (*trans*-phytol) (**9**) (Sims & Pettus, 1976), 7,11,15-trimethyl-2-hexadecanone (**10**) (Worner & Schreier, 1991), β-sitosterol-3-*O*-β-D-glucopyranoside (**11**) (Kojima, Sato, Hatano, & Ogura, 1990) and quercetin 3-*O*-β-D-glucopyranoside (**12**) (Markham, Ternai, Stanley, Geiger, & Mabry, 1978), by comparison of their spectral data with published values. This is the first report for the spectral data of **7**, that was previously prepared synthetically (Churchward, Gibson, Meakins, & Mulley, 1950), and without any reference or spectral evidence isolated from *Semiaquilegia adoxoides* (Feng et al., 2006). The hydroxyl group of compound **7** was confirmed at position 10 by the GC–MS fragmentation pattern. Compound **10** was previously reported in the volatile fraction of *Galium odoratum* (Worner & Schreier, 1991). This is the first report of the isolation of **10** from *N. nucifera* and the first report of its ^{1}H- and ^{13}C NMR data.

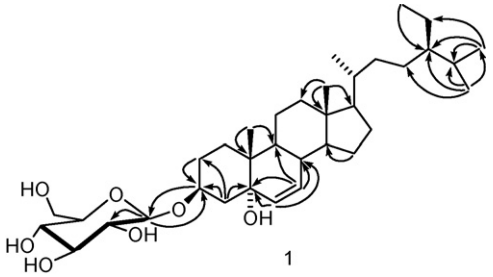

Fig. 2. Key HMBC correlations for compound **1**.

The crude ethanolic extract along with fractions A–D (see Section 3) and all purified compounds except **2** and **6** were evaluated for *in vitro* antimalarial activity (against chloroquine sensitive (D6) and resistant (W2) clones of *Plasmodium falciparum*), cytotoxicity and for antifungal activity. Fractions A, C, D and compounds **3** and **5** exhibited activity against D6 (IC_{50} of 9.2, 3.6, 1.7, 0.2 and 4.8 μg/mL, respectively) and W2 clones (IC_{50} of 3.5, 3.2, 4.5, 0.4 and 4.8 μg/mL, respectively). The selectivity index of the antimalarial activity versus toxicity for compound **3** was 122 and 62 for D6 and W2 clones, respectively, as compared to a selectivity index of 5 for both clones for compound **5**. Chloroquine and artemisinin were used as positive controls which showed IC_{50} values of 16.0 and 8.5 ng/mL (for D6) and IC_{50} of 150.0 and 9.0 ng/mL (for W2), respectively. None of the tested compounds or fractions had cytotoxic effects towards mammalian kidney fibroblasts (Vero cells) up to a concentration of 23.8 μg/mL. Only compound **3** had antifungal activity against *Candida albicans* with IC_{50}/MIC values of 4.5/10 μg/mL, respectively. The positive control amphotericin B gave IC_{50}/MIC values of 0.2/0.6 μg/mL, respectively. This is the first report of the antimalarial activity of **3**.

Compounds **3**–**5** have a similar aporphine alkaloid skeleton. The only difference is substitution at position C-1 and C-2. Compound **4** exhibited no activity and that the most potent metabolite (**3**) possessed a methylenedioxy moiety.

A literature survey for biological activity of **3** and **5** showed that **3** reverses a multidrug resistance phenotype and possessed