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Research Article

O-Benzoquinone and Ester-Linked Hydroxyfatty Acid as Additional Compounds from *Lonchocarpus nicou*

Abstract

New *o*-benzoquinone and a C₁₆ long-chain *para*-coumarate ester were isolated from *Lonchocarpus nicou* roots. Their structural elucidations are based on different spectroscopic data analysis. In addition, the known pterocarpanoids maackiain and flemichapparin-B never reported in *L. nicou* were also isolated along with rotenone, the major phytochemical from this tropical liana.

Rue de la Méditerranéenne, F-34300 Agde. A voucher specimen has been deposited at Laboratoire de Pharmacognosie de Grenoble-Domaine de La Merci, F-38700 La Tronche.

Extraction and isolation

The Air-dried roots (200 g) of *L. nicou* were powdered and extracted by soaking successively at room temperature in 1L of hexane, benzene, chloroform, acetone and methanol for 2 days. The extracts were filtered and the process was repeated twice for each solvent. The filtrates were then combined and concentrated under reduced pressure to give brownish residues. The benzene crude extract (24 g), was then macerated with methanol. After recovery of 16.85 g of rotenone (main compound from *L. nicou*) that constitute the methanol insoluble portion, the soluble part (7.13 g) was subjected to MPLC using SiO₂-Diol column (460 x 26 mm) as stationary phase. 14 Fractions (F1-F14) were collected from this column that was successively eluted with C₆H₁₄ (100%) (F1), C₆H₁₄-EtOAc (8:2) (F2, F3), C₆H₁₄-EtOAc-MeCOEt (7.5:2:0.5) (F4, F5, F6), C₆H₁₄-EtOAc-MeCOEt (7.3:2:1.2) (F7, F8), C₆H₁₄-EtOAc-MeCOEt (65:2.5:1) (F9), C₆H₁₄-CH₂Cl₂-EtOAc-MeCOEt (3.2:3.2:2.5:1.2) (F10), CH₂Cl₂-EtOAc-MeCOEt (6.4:2.6:1) (F11, F12), CH₂Cl₂-EtOAc-MeCOEt (6:3:1) (F13), CH₂Cl₂-EtOAc-MeCOEt (6:1:3) (F14).

F5 fraction (305 mg) was chromatographed again, by eluting with a gradient of C₆H₁₄-EtOAc-MeCOEt mixture, under MPLC with SiO₂ column (460 x 15 mm) giving access to 6 combined fractions (A-F). Fraction A yielded maackiain (7 mg) and Fraction B (14 mg) led to flemichapparin-B (2 mg) after crystallization in MeOH.

F6 fraction (340 mg), after one more MPLC eluted in a gradient way from hexane-Toluene (7:3) to hexane-Toluene-MeOH (0.7:9:0.3) with SiO₂ column (460 x 15 mm) and combination of similar fractions, has furnished 2 combined fractions (A1 and B1). The fraction B1 (17 mg) was then subjected to Sephadex LH-20 column (340 x 15 mm) chromatography [CHCl₃-MeCOEt-MeOH (3:3.5:3.5)] followed by Circular Centrifugal TLC [Toluene to hexane-Toluene-MeOH (0.5:9:0.5)] to yield *o*-benzoquinone (1) (6.7 mg).

Introduction

Extensive chemical investigations of the lipophilic extract of *Lonchocarpus nicou* roots, a tropical liana of the Fabaceae family [1], have led to numerous flavonoids [2-5]. In fact, in earlier publications we reported the isolation of modified B-ring (coumaronochromone) or C-ring rotenoids (*seco*-rotenoids) and hydroxyrotenoids along with others well-known rotenoids [3-5]. In addition to these metabolites, we now wish to report the isolation of two additional compounds: an *o*-benzoquinone and a long alkanolic chain (C₁₆; palmitic acid) ester of *para*-coumaric acid. Their chemical structures were established by spectroscopic methods UV, MS as well as 1D and 2D NMR. The present work aims to achieve better understanding phytochemical composition of *L. nicou*, since new families of compounds were isolated (quinone, *p*-coumaric acid derivative and pterocarpanoids).

Materials and Methods

General experimental procedure

MPLC was conducted using Büchi 681 pump (max. pressure = 40 bars) connected to Büchi columns with different sizes (460 x 26 mm; 460 x 15 mm; 230 x 15 mm) and stationary phases such as: SiO₂-diol (Merck LiChroprep® DIOL, 15-40 μm), SiO₂ (Merck LiChroprep® Si 60, 15-25 μm) and polyamide (Macherey-Nagel SC-6, < 70 μm). Circular Centrifugal TLC were performed on Chromatotron® apparatus (model 8924 Harrison Research) equipped with RHSY pump model. NMR spectra were recorded on Bruker DPX Avance 400 MHz at 298 K. Chemical shifts were expressed in δ (ppm) referring to TMS for both ¹H and ¹³C NMR experiments. Characteristic NMR resonances were assigned on the basis of 1D and 2D experiments: COSY, HMQC, HMBC and NOESY. HRESMS spectra were performed on a Q-TOF System spectrometer.

Plant material

L. nicou roots (1 kg) were obtained as a gift from the firm Sedagri-

F10 fraction (251 mg) was one more subjected to MPLC with SiO₂ column (460 x 15 mm) by using a gradient of C₆H₁₄-EtOAc-MeCOEt mixture. This purification process led to 3 combined fractions (A'-C'). The purification of fraction A' (24 mg) was further realized by using another MPLC with polyamide column (230 x 15 mm) [gradient from hexane to CH₂Cl₂-MeCOEt-MeOH (5:2.5:2.5)] leading to 16-(E)-*p*-coumaroyloxypalmitic acid (2) (13.4 mg).

(-)-Maackian (3): Physical and spectral data are in accordance with those previously published in literature [6-8].

(-)-Flemichapparin-B (4): Physical and spectral data are in accordance with those previously published in literature [9,10].

(-)-Rotenone (5): Physical and spectral data are in accordance with those previously published in literature [11,12].

Results and Discussion

From benzene extract of *L. nicou* roots, two new compounds 1 and 2 were isolated together with the known maackian (3), flemichapparin-B (4) and rotenone (5) as described in Materials and Methods. Chemical structures of new compounds were elucidated as follows.

Compound 1 was isolated as a yellow needle which exhibited an intense UV band at λ_{max} (MeOH): 288 and 408 nm respectively, supposing a large conjugated system. The molecular formula C₈H₈O₄ was deduced from HRESMS (found: 169.0491; calcd: 169.0501 for [M+H]⁺). The ¹³C NMR data display only four signals respectively at δ 178.9; 163.7; 103.0 and 57.1, suggesting a symmetric compound. Indeed, δ 178.9 was assigned to a carbonyl group whereas δ 163.7 was attributed as *O*-bonded quaternary sp² C. Moreover, a shielded double bond carbon appears at δ 103.0 as well as a methoxy group at δ 57.1. ¹H NMR analysis is also in accordance with a symmetric compound since only two signals were observed as singlets: δ 5.77 (δ 103.0) and δ 3.90 (δ 57.1). Therefore, two dimethoxybenzoquinone structures are possible in keeping with ¹H, ¹³C, HMQC and HMBC

NMR analysis: 2,5-dimethoxy-*p*-benzoquinone and 4,5-dimethoxy-*o*-benzoquinone. Further, the correlation observed in NOESY experiment led to elucidation of final structure unequivocally assigned as 4,5-dimethoxy-*o*-benzoquinone (1) for which the trivial name of lonchoquinone was proposed.

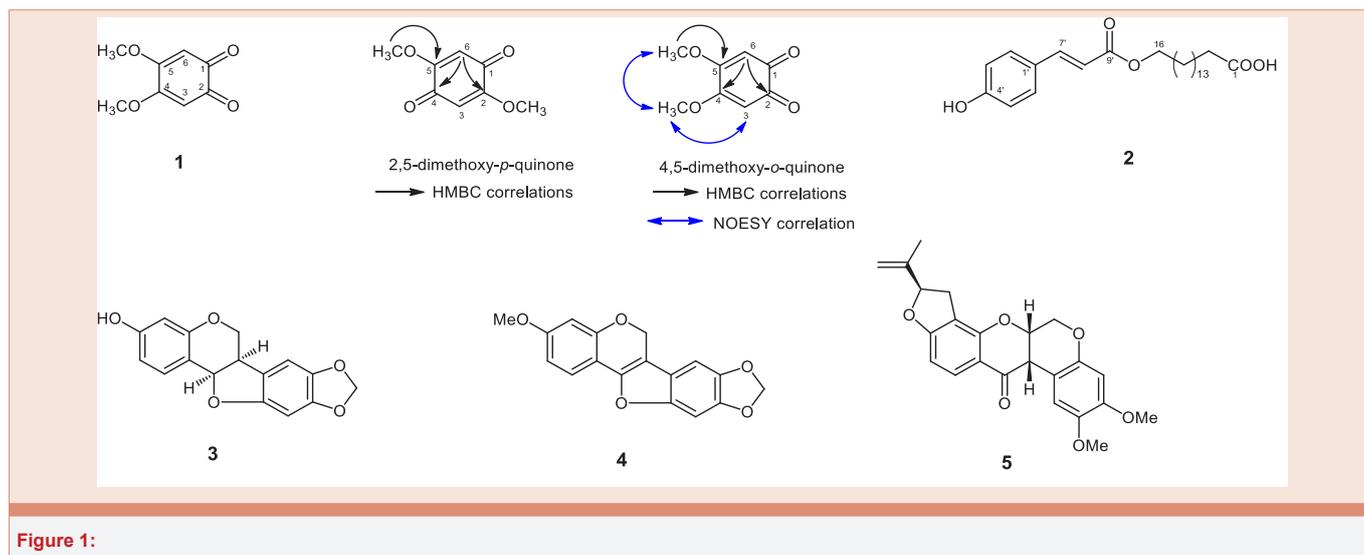
Compound 2 was isolated as a colourless amorphous solid which exhibited a UV band at λ_{max} (MeOH): 242, 298 and 387 nm. The molecular formula was determined as C₂₅H₃₈O₅ by HRESMS (found: 441.2630; calcd: 441.2617 for [M+Na]⁺). The ¹H NMR data clearly showed two different partial structures: a *para*-coumaric acid moiety and a long carboxyalkyl chain. In fact, aromatic *ortho*-coupled (*J* = 7.6 Hz) protons as displayed in ¹H NMR data at δ 7.41 (2H) and δ 6.84 ppm (2H), suggested a 1,4-disubstituted benzene ring. Whereas, a hydroxyl group (δ 6.25 ppm) was placed at position 4, the other position (*para*) was supposed to be linked to conjugated ethylenic double bond involving a cinnamic acid derivative. These two ethylenic protons observed at δ 7.62 and δ 6.25 ppm were assigned as *trans*-configuration as supported by the coupling constant (*J* = 15.9 Hz). The ¹³C NMR data was in agreement with *para*-coumaric acid moiety with 9 sp² atoms (167.8 ppm < δ < 115.5 ppm): One ester carbonyl group C-9' (167.8 ppm), One *O*-substituted and C-substituted quaternary carbon, respectively C-4' (158.0 ppm) and C-1' (127.0 ppm), Four aromatic carbons C-2' and C-6' (129.9 ppm) and C-3' and C-5' (115.9 ppm) and finally two ethylenic carbons C-7' (144.5 ppm) and C-8' (115.5 ppm).

In the aliphatic proton region, the ¹³C NMR spectrum showed 15 CH₂ (64.7 ppm < δ < 26.0 ppm) and one terminal carboxylic group C-1 (δ 178.5 ppm). Moreover, ¹H NMR spectrum displayed two triplets with the same intensity at δ 4.20 ppm (*J* = 6.6 Hz) and δ 2.34 ppm (Table 1), (Figure 1).

(*J* = 7.2 Hz) corresponding to two terminal methylene groups CH₂-16 (δ 64.7 ppm) and CH₂-2 (δ 33.8 ppm), respectively, attached to the ester linkage and to carboxylic acid group. The remaining CH₂

Table 1: ¹H (400 MHz) and ¹³C (100 MHz) NMR data for 4,5-dimethoxy-*o*-benzoquinone (1) and 16-(E)-*p*-coumaroyloxypalmitic acid (2) in CDCl₃ (δ ppm; J Hz).

1			2		
H/C	¹³ C	¹ H	H/C	¹³ C	¹ H
1,2	178.9		1	178.5	
3,6	103.0	5.77 s	2	33.8	2.34 br t (7.2)
4,5	163.7		3	26	1.63 m
OCH ₃	57.1	3.90 s	4-14	29.5 (x2)	1.29 m
				29.4 (x6)	
				29.2 (x2)	
				29.0	
			15	28.7	1.67 m
			16	64.7	4.20 t (6.6)
			1'	127	
			2',6'	129.9	7.41 d (7.6)
			3',5'	115.9	6.84 d (7.6)
			4'	158.0	
			7'	144.5	7.62 br d (15.9)
			8'	115.5	6.25 d (15.9)
			9'	167.8	
			4'-OH		6.55 s



were displayed as multiplet at δ 1.67 ppm (2H), δ 1.63 ppm (2H) and δ 1.29 ppm (22H). Finally, the aliphatic part of this compound was identified as a C_{16} fatty acid (palmitic acid). Consequently, the above described compound was assigned as a new natural product named 16-(E)-*p*-coumaroyloxypalmitic acid (2). This compound probably resulted from esterification between the widespread *para*-coumaric acid and ω -hydroxypalmitic acid. To the best of our knowledge, except the *para*-coumaric acid methyl ester (C_2 alkyl chain) isolated in *Lonchocarpus xuul* [13], alkyl *para*-coumarates are quite rare in *Lonchocarpus* genus. However, long-chain alkyl *para*-coumarates (C_n saturated or unsaturated) [14–18], are more frequently encountered in plant kingdom than carboxyalkyl *para*-coumarates derivatives even though a series of (E)-*para*-coumaroyl esters of ω -hydroxyfattyacids (C_{24} to C_{28}) were already identified in minor amounts in the leaf fiber of *Musa textilis* [19] without any NMR data.

Conclusion

In summary, we report herein the first isolation of 4,5-dimethoxy-*o*-benzoquinone (1) and 16-(E)-*p*-coumaroyloxypalmitic acid (2) as natural products. Additionally to these newly reported compounds, two known pterocarpanoids (maackiain and flemichapparin-B) as well as the pivotal compound rotenone were also isolated from *L. nicou* roots. It is important to highlight the fact that this is the first isolation of these two known pterocarpanoids from *L. nicou*. Undoubtedly, this study constitutes a major contribution to the phytochemical investigation of *L. nicou* roots which is rotenoid-yielding plant as showed by previous studies.

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