ISOLATION, FOR THE FIRST TIME, OF A FLAVONOID GLYCOSIDE AND THE (±)-CATECHIN FROM THE AERIAL PARTS OF *EBENUS PINNATA*

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(Reçu le 18 Février 2003, accepté le 23 Juin 2004)

ABSTRACT: From the aerial parts of *Ebenus pinnata*, a flavonol diglycoside and a mixture of two flavan-3-ol enantiomers were isolated for the first time and their structures elucidated as quercetin 3-*O*-α-L-rhamnosyl-(1→6)-*O*-β-D-glucopyranoside **1**, and (±)-catechin **2**. Their structures were established by ESIMS, EIMS, and 1D and 2D NMR spectral analysis.

Key words: *Ebenus pinnata*, Leguminosae, (±)-catechin, favonol glycoside, rutin, NMR.

RESUME : De la partie aérienne de la plante *Ebenus pinnata*, un flavonol diglycoside et un mélange de deux flavan-3-ol énantiomères ont été isolés pour la première fois et identifiés comme la quercetine 3-*O*-α-L-rhamnosyl-(1→6)-*O*-β-D-glucopyranoside **1** (rutine) et la (±)-catechine **2**. Leurs structures ont été confirmées à l'aide de la spectrométrie de masse ESIMS, EIMS et de quelques techniques spectroscopiques en RMN 1 et 2D.

Mots clés: *Ebenus pinnata*, Legumineuse, (±)-catechine, favonol glycoside, rutine, RMN.

INTRODUCTION

The present research is one of our contributions to the biological and chemical studies of medecinal plants growing in Tunisia [1-12]. *Ebenus pinnata* Ait. is endemic to North Africa (Tunisia, Algeria and Morocco). No phytochemical investigation has previously been recorded on this species. We remind that *Ebenus pinnata* belonging to the Leguminosae family is widespread in the region of Chott Mériem (Sousse, Tunisia). We report in this paper the isolation and the structure elucidation of quercetin 3-*O*-α-L-rhamnosyl-(1→6)-*O*-β-D-glucopyranoside (rutin) **1** and a mixture of (±)-catechin **2** from the ethyl acetate extract of the aerial parts of *E. pinnata*. Their structures were established on the basis of 2D NMR spectroscopic experiments. We remind that rutin **1** and catechin **2** are phenolic substances very known by their various biological activities including antiallergenic, antiviral, anti-inflammatory, and vasodilating actions. However, most interest has been devoted to the antioxidant activity of these type of compound, which is due to their ability to reduce free radical formation and to scavenge free radicals [13]. Other previous investigations shown that the presence of catechin skeleton in some compound structures induced an anti-inflammatory effect [14]. The combination of rutin with other flavonoids exhibited much more antibacterial activity against *Bacillus cereus* and *Salmonella enteritidis* than either flavonoid alone [15]. Rutin **1** has also shown an important anti-inflammatory effect [16].

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RESULTS AND DISCUSSION

Compound **1** was obtained as yellow amorphous powder. The positive ion ES mass spectrum of **1** (Figure 1) exhibited pseudomolecular ions $[M + H]^+$and $[M + Na]^+$ at *m/z* 611 and 633, respectively, which were compatible with the molecular formula $C_{27}H_{30}O_{16}$. The ^1H and ^{13}C NMR spectra of **1** display characteristic signals for a quercetin moiety and two sugars residues. The ^{13}C NMR spectrum showed 27 resonances, deduced from DEPT and HMQC experiments into 1 CH_3, 1 CH_2, 15 CH and 10 quaternary C. In the ^1H NMR spectrum are two coupled doublets, at δ_H 6.25 (d, J = 2.1 Hz) and 6.44 (d, J = 2.1 Hz), typical of two *meta*-related H-6 and H-8 protons of flavonoid unit ring. The ABM spin system of B-ring δ_H 7.69 (d, J = 2.1 Hz), 7.66 (dd, J = 8.4, 2.1 Hz) and 6.92 (d, J = 2.1 Hz) permits the identification of the aglycon as quercetin [17-19]. On the basis of chemical shifts, multiplicity, and the coupling constants values of anomeric protons δ_H 5.13 (d, J = 7.8 Hz) and 4.56 (d, J = 1.2 Hz), sugar residues were identified as β-glucopyranose, and α-rhamnopyranose, respectively (Table 1). The chemical shifts of C-2 (δ_C 159.4) and C-3 (δ_C 135.6) indicated the substitution at the C-3 of the quercetin moiety. The HMBC experiment (Figures 2 and 3), which displayed correlation between the anomeric proton of β-glucopyranose (δ_H 5.13, H-1″) and C-3 (δ_C 135.6) of the quercetin aglycon, confirmed the glycosidation site to he at C-3. A long-range correlation was also observed between H-1‴ (δ_H 4.56) of rhamnose and C-6″ (δ_C 68.6) of glucopyranose, and vice versa, indicating the interglycosidic linkage of disaccharide residue. On the basis of these results, the structure of **1** was elucidated as quercetin-3-*O*-α-L-rhamnosyl-(1→6)-*O*-β-D-glucopyranoside **1** (rutin).

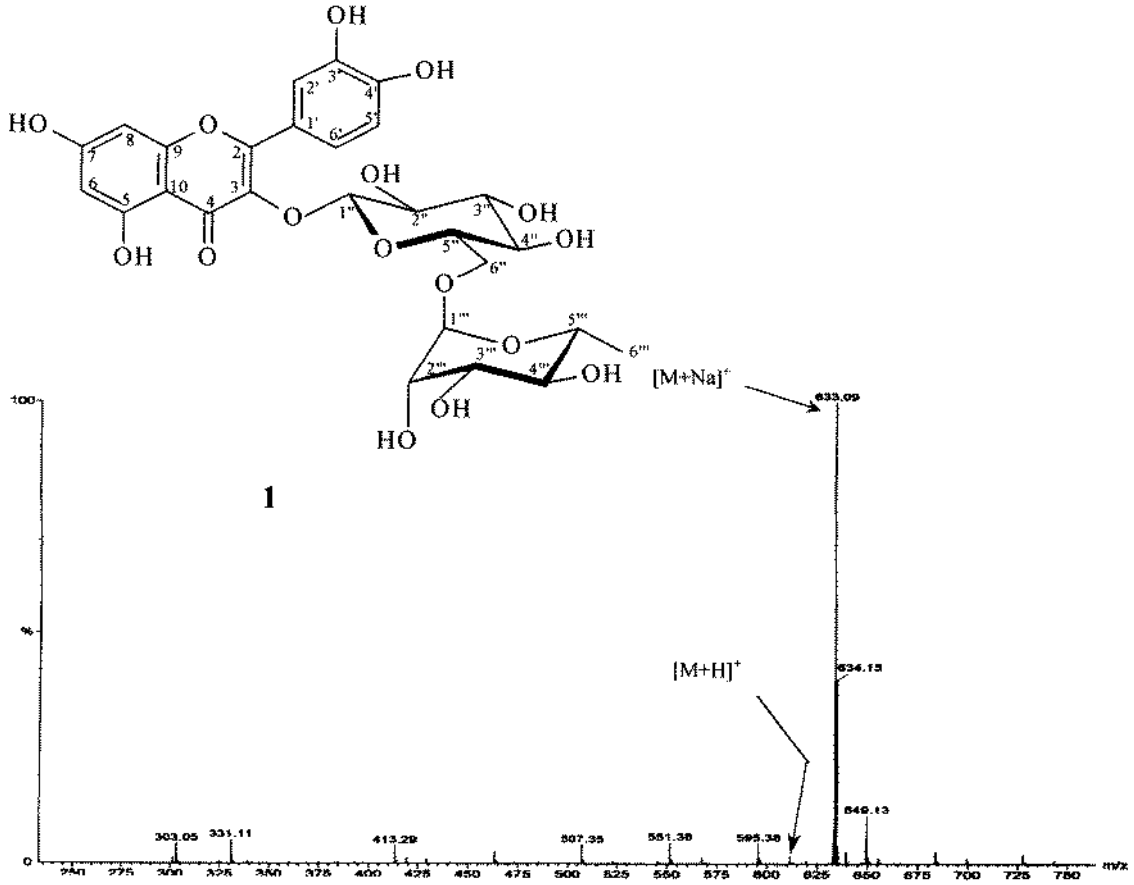

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Figure 1. Positive ion ES mass spectrum of compound 1.

Table 1: ^{1}H NMR and ^{13}C NMR for compound 1 (^{13}C, 125 MHz ; ^{1}H, 500 MHz, CD_3OD)

Position	C atom	^{13}C	^{1}H, *J* (Hz)
flavonol			
2	C	159.4	
3	C	135.6	
4	C	179.4	
5	C	162.9	
6	CH	100.0	6.25 d (1.2)
7	C	166.1	
8	CH	94.9	6.44 d (1.2)
9	C	158.5	
10	C	105.6	
1'	C	123.1	
2'	CH	117.7	7.69 d (2.1)
3'	C	145.8	
4'	C	149.8	
5'	CH	116.1	6.92 d (2.1)
6'	CH	123.6	7.66 dd (8.4, 2.1)
glucose			
1''	CH	104.6	5.13 d (7.8)
2''	CH	75.7	3.52 d d (7.9, 10.4)
3''	CH	78.1	3.47
4''	CH	69.7	3.48
5''	CH	77.4	3.38
6''	CH_2	68.6	3.38
			3.84 br d (10.8)
rhamnose			
1'''	CH	102.4	4.56 d (1.2)
2'''	CH	72.1	3.67 dd (1.5, 3.3)
3'''	CH	72.2	3.56 dd (3.3, 9.3)
4'''	CH	73.9	3.30 t (9.6)
5'''	CH	71.4	3.35 dq (9.6, 6)
6'''	CH_3	17.9	1.16 d (6.1)

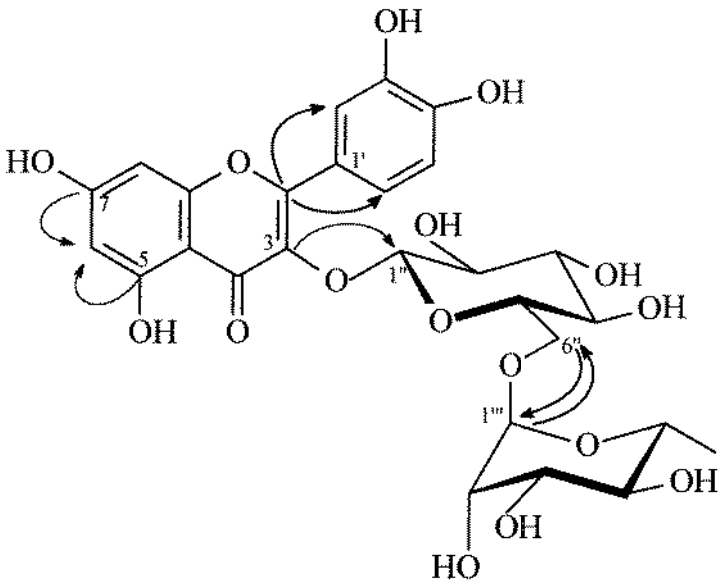


Figure 2. Heteronuclear multiple-bond correlations for 1. Arrows point from carbon to proton.

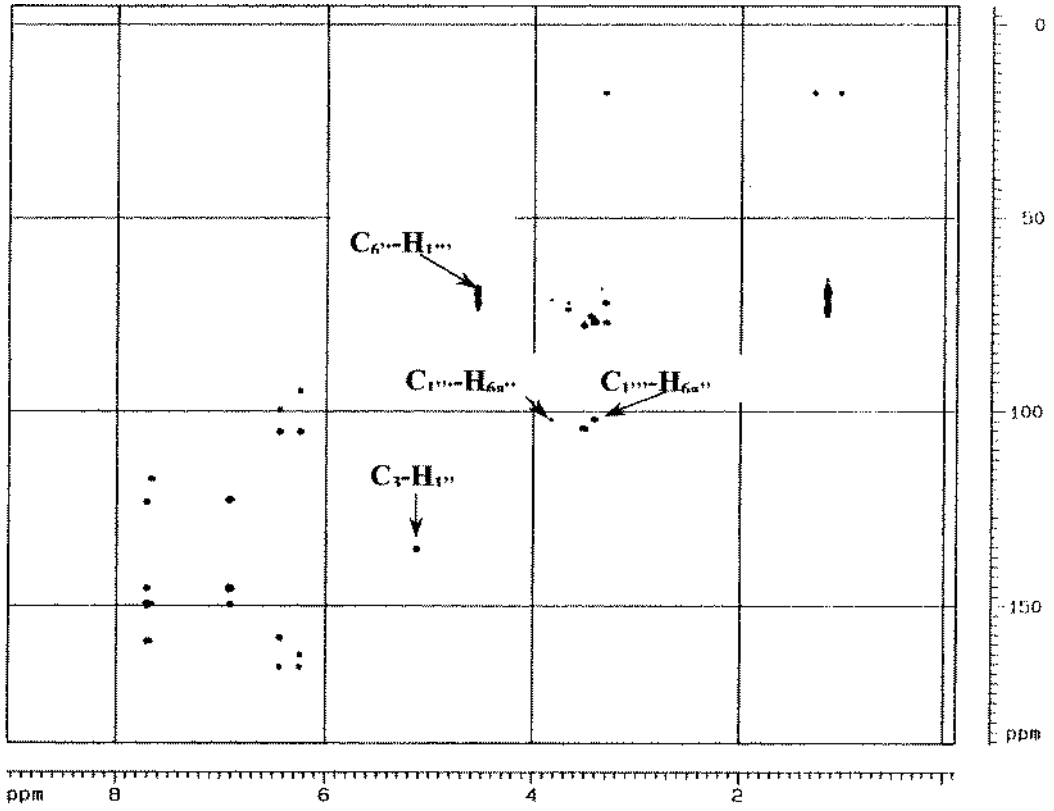
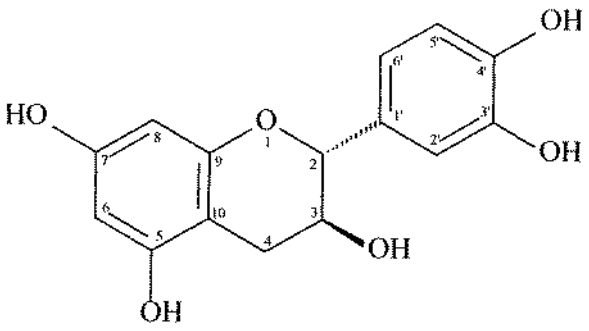
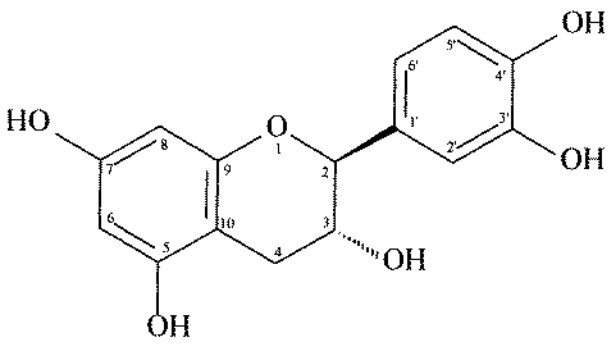


Figure 3. HMBC spectrum of compound 1.

Compounds **2(+)** and **2(-)** were obtained together as yellow amorphous powder. Their same molecular formula was determined to be $C_{15}H_{14}O_6$ on the basis of the EI mass spectrum (*m/z* 290 $[M]^{+\cdot}$) (Figure 4). The ^1H NMR spectrum (Table 2) showed many similarities to **1** revealing *meta*-coupling aromatic protons signals for H-6 5.98 (d, J = 2.4) and H-8 5.90 (d, J = 2.1) and an ABM spin system at δ_H 6.88 (d, J = 1.5), 6.81 (d, J = 8.1) and 6.76 (dd, J = 8.1, 2.1). The chemical shift values and the coupling constants of the protons at δ_H 2.55 (dd, J =18.9, 8.1), 2.90 (dd, J = 15.9, 5.4), 4.02 (td, J = 15.9, 5.4) and 4.61 (d, J = 7.5) suggested the presence of the -O-CH-CH-CH_2- system in the C-ring. The connectivities of the molecular fragments were established by the heteronuclear multiple bond correlation experiment (HMBC) (Figures 5 and 6), showing long-range correlation between C_{10}-H_6, C_{10}-H_8, C_{10}-H_3, C_{10}-H_{4a}, C_{10}-H_{4b}, C_9-H_2, C_2-$H_{2'}$ and C_2-$H_{6'}$. The optical rotation of $[\alpha]^{23}_D$ -5,4 (*c*1.37, MeOH) when compared to reported data of –14.3 [20] reveals the presence of a mixture of (±)-catechin **2**.



2(+)
(+)-catechin



2(-)
(-)-catechin

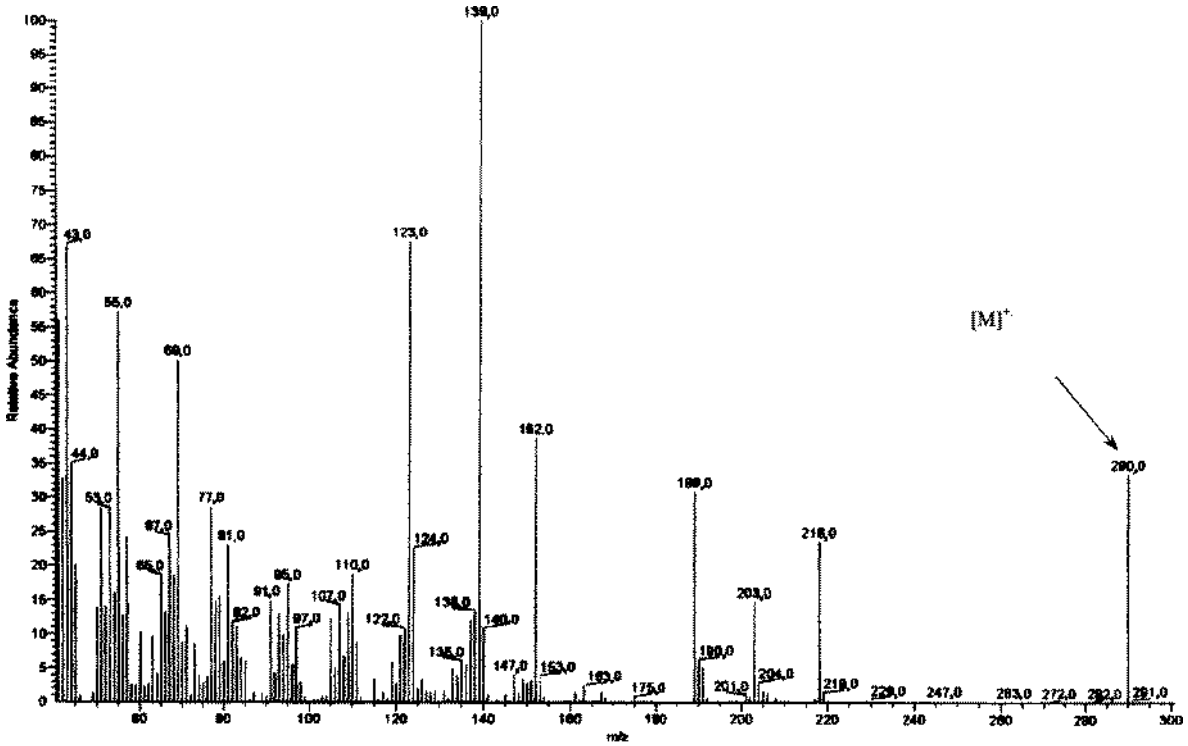


Fig. 4. EI mass spectrum of compound 2(±).

Table 2: ^{1}H NMR and ^{13}C NMR for compound 2(±)
(^{13}C, 75 MHz ; ^{1}H, 300 MHz, CD_3OD)

Position	C atom	^{13}C	^{1}H, *J* (Hz)
2	CH	83.3	4.61 d (7.5)
3	CH	69.2	4.02 td (7.8, 5.4)
4	CH_2	28.9	2.90 dd (15.9, 5.4)
			2.55 dd (15.9, 8.1)
5	C	158.0	
6	CH	96.7	5.98 d (2.4)
7	C	158.2	
8	CH	95.9	5.90 d (2.1)
9	C	157.3	
10	C	101.2	
1'	C	132.6	
2'	CH	115.7	6.88 d (1.5)
3'	C	146.2	
4'	C	146.2	
5'	CH	116.5	6.81 d (8.1)
6'	CH	120.4	6.76 dd (8.1, 2.1)

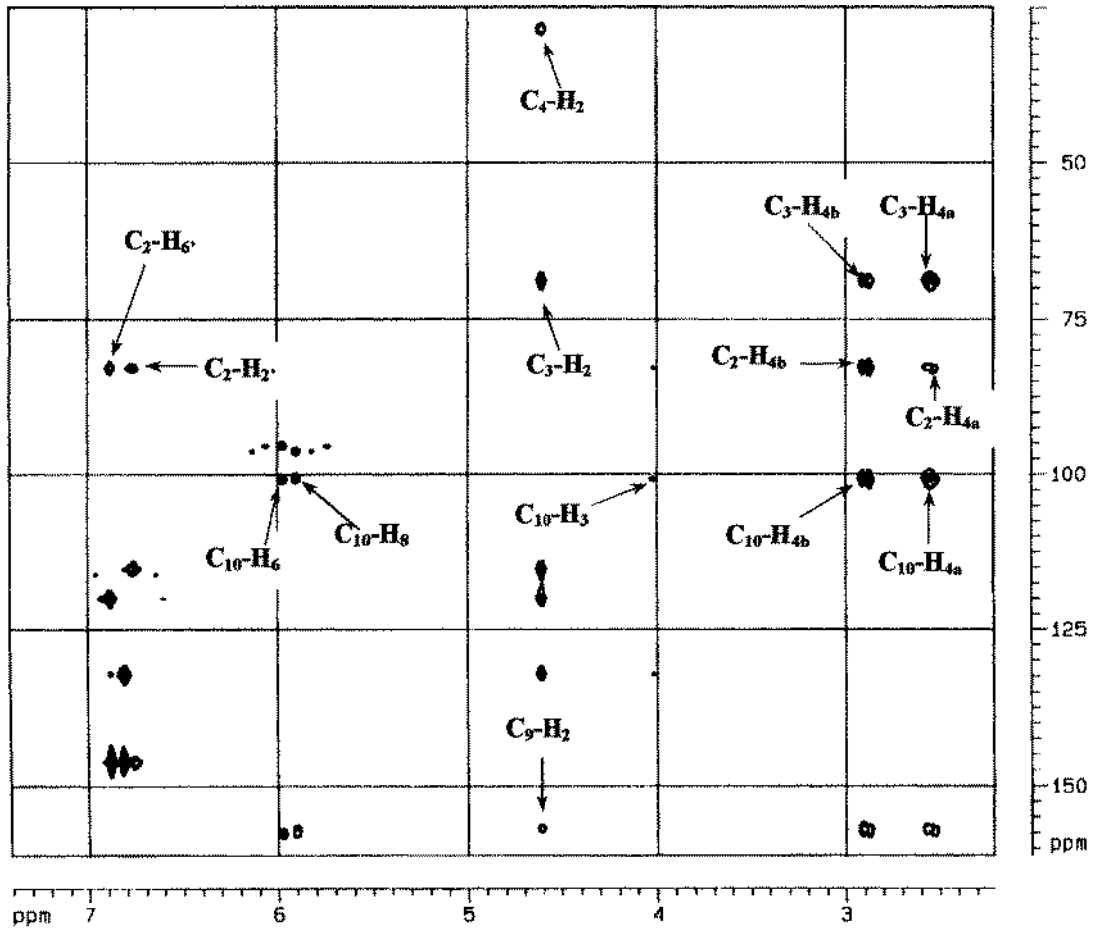


Fig. 5. HMBC spectrum of compound 2(±).

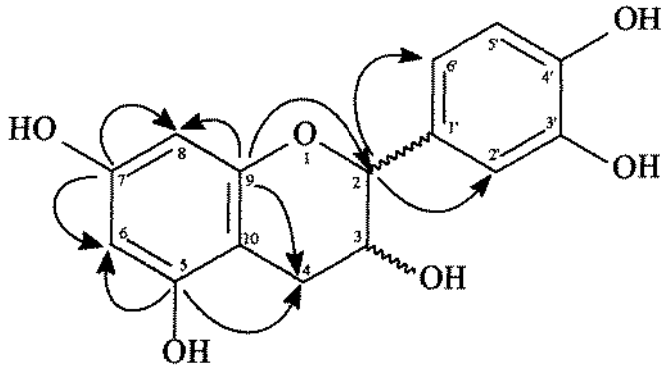


Fig. 6. Heteronuclear multiple-bond correlations for (2±).
Arrows point from carbon to proton.

EXPERIMENTAL SECTION

General Experimental procedures

Melting points were determined on an electrotherma 9100 micro melting point apparatus. Optical rotation was measured with a Jasco DIP-300 polarimeter. ^{1}H and ^{13}C spectra were obtained with a Bruker NMR-300 spectrometer, operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C, and a Bruker AMX-500 spectrometer operating at 500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C. All NMR spectra were obtained in CD_3OD, with chemical shifts (δ) expressed in ppm and coupling contants (J) in Hz. HMBC, HMQC experiments were carried out using a Bruker AMX-500 spectrometer.

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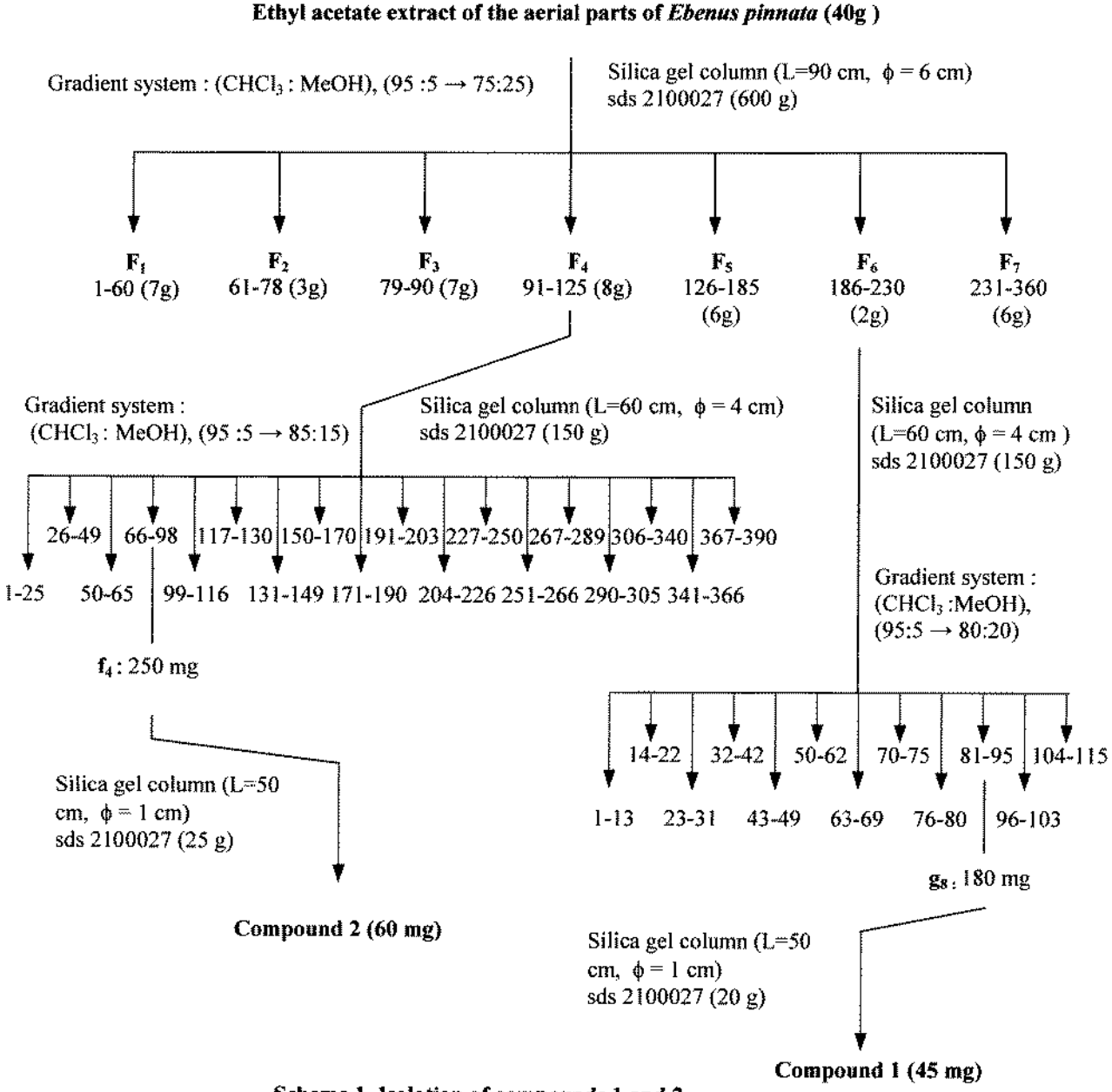
Plant material.

Ebenus pinnata Ait. (Liguminosae) was collected from Chott Mériem Sousse (Tunisia), in May 1999. A voucher specimen has been deposited in the herbarium of the Ecole Supérieure d'Horticulture et d'Elevage de Chott Mériem, Université du Centre, Sousse, Tunisie.

Extraction and isolation.

Dried and finely powdered *Ebenus pinnata* aerial parts (1500g) was extracted with petroleum ether (40-60°C), methylene chloride, acetone, methanol and water successively using a soxhlet. Methanol extract was filtered then evaporated to dryness. The crude extract (120 g) was suspended in water and partitioned with chloroform and ethyl acetate yielding 10 g and 40 g, respectively.

The following flow-chart describes the isolation procedures of quercetin 3-*O*-α-L-rhamnosyl-(1→6)-*O*-β-D-glucopyranoside **1** (quercetin-3-O-rutinoside) and the (±)-catechin **2** (Scheme 1).



Scheme 1. Isolation of compounds 1 and 2.

Compound 1: yellow amorphous powder; mp 196-198°C; positive ion ESIMS *m/z* 611 $[M + H]^+$, 633 $[M + Na]^+$; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD), see Table 1.
Compound 2(±): yellow amorphous powder; $[\alpha]^{23}_D$ -5,4 (*c* 1.37, MeOH); EI-MS *m/z* $[M]^{+\cdot}$ 290 (33), 218 (23), 303 (14), 189 (31), 152 (38), 139 (100), 124 (22) ; 123 (67) ; ^1H NMR (300 MHz, CD_3OD) and ^{13}C NMR (75 MHz, CD_3OD), see Table 2.

AKNOWLEGMENT: The authors wish to thank Dr. F. Harzallah-Skhiri (Ecole Supérieure d'Horticulture et d'Elevage de Chott Mériem, Université du Centre, Sousse, Tunisie) for botanical classification of the plant material.

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