

## FLAVONOIDAL CONSTITUENTS OF THE FLOWERS OF *ACACIA SALIGNA*

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### ABSTRACT

From the flowers of *A. saligna* a new flavanone glycoside (naringenin 7-O- $\beta$ -D-glucoside 6''-acetate) was isolated, besides three known flavanones (naringenin, naringenin 7-O- $\beta$ -D-glucoside and 6-C-glucosylnaringenin). Two flavonols (quercetin and quercitrin),  $\beta$ -sitosterol and  $\beta$ -sitosterol-O-glucoside. Their structures were determined based on their spectral data (IR, UV, EI MS, FAB MS,  $^1\text{H}$ - and  $^{13}\text{C}$  NMR). Assignments of the  $^{13}\text{C}$  NMR were confirmed by 2D NMR experiments and addressed herein for the first time for some of these compounds.

### INTRODUCTION

*Acacia saligna* is a low tree or tall shrub belonging to the family Leguminosae.<sup>(1)</sup> The genus *Acacia* comprises about 1200 species<sup>(2)</sup>, dispersed throughout the tropics and to some extent in the temperate regions<sup>(1)</sup>. Many species of *Acacia* have been described to have astringent, aphrodisiac, anti-ulcer and antisiphilitic properties.<sup>(3,4)</sup> *Acacia* plants have been used in the treatment of diarrhea, gynecological diseases, hemorrhage and leprosy, as well as sedative in labour, abortifacient and as antimicrobial<sup>(4,5)</sup>. More recent reports pointed out the CNS depressant, spermicidal and filaricidal activities<sup>(6)</sup>. The molluscicidal properties of *Acacia* species have also been reported<sup>(7)</sup>, and were attributed mainly to their flavonol and tannin contents<sup>(10)</sup>.

Previous phytochemical studies have shown that the genus *Acacia* elaborated a variety of interesting secondary metabolites viz. triterpenoid saponins<sup>(8,11)</sup>, alkaloids<sup>(12)</sup>, tannins<sup>(5)</sup>, cyanogenetic glycosides<sup>(13)</sup>, anthraquinones<sup>(14)</sup> and different flavonoids<sup>(3,5,10,15-30)</sup>. Other published papers were concerned with the chemical composition of the gum exudates<sup>(31,32)</sup> and essential oils<sup>(33,34)</sup> of *Acacia* plants.

Despite the wealth of information describing the different flavonoids obtained from *Acacia* species (flavones<sup>(15,16)</sup>, chalcones<sup>(17,19,30)</sup>, flavonols<sup>(3,10,20-22)</sup>, flavanones<sup>(17,21,23)</sup>, isoflavones<sup>(20)</sup>, catechins<sup>(15,24,25)</sup>, dihydroflavonols<sup>(21,25)</sup>, flavans<sup>(26)</sup>, flavan dimers<sup>(27,28)</sup>, biflavonols<sup>(15)</sup>, auronols<sup>(29)</sup> and leucoanthocyanidins<sup>(15)</sup>], no reports concerning the flavonoidal constituents of *A. saligna* could be found in the literature. This beside the previously mentioned medicinal uses and biological activities of *Acacia* plants, aroused the interest to investigate the flavonoidal constituents of the flowers of *A. saligna* cultivated in Egypt.

This paper describes the isolation and structure determination of four flavanones: naringenin (1), naringenin 7-O- $\beta$ -D-glucoside (4) and its 6''-acetate ester (3) besides the rare C-glycosylflavanone (5). In

addition, quercetin (2), quercitrin (6),  $\beta$ -sitosterol and  $\beta$ -sitosterol-O-glucoside were also isolated.

### EXPERIMENTAL

#### General:

Melting points were measured on a Buchi B-521 apparatus (Switzerland) and were not corrected. UV spectra were recorded on a UV-Visible recording spectrophotometer (Shimadzu UV-260, Japan). IR spectra were recorded on a Pu-9706 IR spectrophotometer (Philips, England).  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were determined with a Bruker AC-300, at 300 and 75 MHz, respectively; chemical shifts are given in ppm with TMS as internal standard; a series of NMR experiments (APT, DEPT and HETCOR) aided assignments. EI MS were measured on a Varian MAT 311A spectrometer operating at 70 eV. FAB MS were determined on Ion Tech 11NF, in the positive ion mode using glycerol matrix and Xe as fast atom beam. Silica gel 60 (Merck) was used for CC and pre-coated TLC plates (Merck) were used.

#### Plant material:

The yellow flowers of *Acacia saligna* Wendl (Leguminosae) were gathered from plants cultivated alongside the Cairo-Bulbis desert road, near Bulbis city, Egypt; in March - April 1996. Identification was kindly confirmed by Dr. N. El-Hadidi, Prof. of Taxonomy, Faculty of Sciences, Cairo University (who is here acknowledged). A voucher specimen was kept in the Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt.

#### Extraction and isolation of constituents:

The dried and crushed flowers of *A. saligna* (2 kg), were extracted by maceration with ethyl alcohol 95% (6L X 3: 3 days), at room temperature, and the alcoholic extract was concentrated to a yellowish-brown semi-solid (260 g). The later residue was then extracted with boiling distilled water (1.5 L), filtered and the filtrate was repeatedly extracted with ether (0.5 L X 5), then with ethyl acetate (0.5 L X 5). The ether fraction (11 g) was chromatographed on silica gel

column (60 X 3.5 cm), using benzene with increasing amounts of MeOH. Fractions eluted with 2% MeOH gave 45 mg of  $\beta$ -sitosterol, as colorless crystals, mp 139° (CHCl<sub>3</sub>-MeOH); fractions eluted with 4% MeOH provided compound 1 (35 mg), and fractions eluted with 6% MeOH gave 65 mg of  $\beta$ -sitosterol-O-glucoside, as a white powder, mp 280-283° (MeOH). The previously obtained ethyl acetate fraction (68 g) was further fractionated on a column of silica gel (90 X 7 cm) using CHCl<sub>3</sub> with increasing proportions of MeOH to give three main fractions I-III. Fraction I (4% MeOH) furnished 25 mg of 2 upon repeated crystallisation from MeOH, fraction II (8% MeOH), was rechromatographed over a column of Si gel using a mixture of benzene-acetone-MeOH (45:45:10) to give 3 (75 mg). Fraction III (10% MeOH), was further chromatographed in a similar way as fraction II to provide 4 (155 mg), followed by a mixture of 5 and 6. This mixture was separated by repeated CC using Si gel and mixture of CHCl<sub>3</sub>-MeOH (85:15) to give 5 (115 mg) and 6 (85 mg).

**Compound 1:** Colorless microneedles, mp 252-3° (CHCl<sub>3</sub>-MeOH). IR  $\nu^{KBr}$  cm<sup>-1</sup>: 3200-3350, 1645, 1610, 1500, 1470 and 1360. UV  $\lambda_{max}$  nm, MeOH: 289, 330(sh); +NaOMe: 247, 324; MeOH + AlCl<sub>3</sub>: 310, 377; MeOH + AlCl<sub>3</sub>+HCl: 309, 375; MeOH + NaOAc: 285(sh), 324, MeOH + NaOAc+H<sub>3</sub>BO<sub>3</sub>: 289, 324(sh). EI MS m/z (rel. int. %): 272[M]<sup>+</sup> (90), 271(39), 179(30), 178(8), 166(28), 153(100), 152(23), 124(19), 120(84), 119(17), 107(22) and 91(21). <sup>1</sup>H- and <sup>13</sup>C NMR: Tables 1 and 2, respectively.

**Compound 2:** Yellow microneedles, mp 300° (char. MeOH). IR  $\nu^{KBr}$  cm<sup>-1</sup>: 3300-3500, 1645 and 1520. UV  $\lambda_{max}$  nm, MeOH: 256, 270(sh), 303(sh), 373; +NaOMe: 248(sh), 295(sh), 331; MeOH + AlCl<sub>3</sub>: 270, 300(sh), 447; MeOH + AlCl<sub>3</sub>+HCl: 266, 300(sh), 360, 428, MeOH + NaOAc: 274, 326, 401; MeOH + NaOAc+H<sub>3</sub>BO<sub>3</sub>: 260, 298(sh), 390. EI MS m/z (rel. int. %): 302[M]<sup>+</sup> (100), 301(17), 273(7), 153(9), 137(13) and 128(9). <sup>1</sup>H- and <sup>13</sup>C NMR: Tables 1 and 2, respectively.

**Compound 3:** Colorless needles, mp 218° (CH<sub>2</sub>Cl<sub>2</sub>-MeOH). IR  $\nu^{KBr}$  cm<sup>-1</sup>: 3200-3500, 1735, 1665, 1620, 1475, 1375 and 1270. UV  $\lambda_{max}$  nm, MeOH: 225, 282, 310(sh), +NaOMe: 248, 324; MeOH + AlCl<sub>3</sub>: 225, 280(sh), 309, 376; MeOH + AlCl<sub>3</sub>+HCl: 224, 280(sh), 308, 377; MeOH + NaOAc: 251, 280(sh), 325; MeOH + NaOAc+H<sub>3</sub>BO<sub>3</sub>: 282, 320(20). EI MS m/z (rel. int. %): 272[M-glucosyl acetate]<sup>+</sup> (84), 271(33), 179(27), 166(25), 153(100), 152(19), 124(17), 120(67), 107(18), 91(18) and 69(26). <sup>1</sup>H- and <sup>13</sup>C NMR: Tables 1 and 2, respectively.

**Compound 4:** Colorless needles, mp 221-3° (MeOH). IR  $\nu^{KBr}$  cm<sup>-1</sup>: 3300-3500, 1660, 1610, 1590, 1500,

1455 and 1370. UV  $\lambda_{max}$  nm, MeOH: 226, 281, 310(sh); +NaOMe: 248, 323; MeOH + AlCl<sub>3</sub>: 227, 281, 310(sh), 359; MeOH + AlCl<sub>3</sub>+HCl: 225, 279, 460(sh); MeOH + NaOAc: 253, 283, 324; MeOH + NaOAc+H<sub>3</sub>BO<sub>3</sub>: 232, 283, 215(sh). EI MS m/z (rel. int. %): 272[M-sugar]<sup>+</sup> (77), 271(34), 179(26), 166(26), 153(100), 152(20), 124(16), 120(74), 119(14), 107(21), 91(19) and 69(25). FAB MS: 435[M+1]<sup>+</sup>. <sup>1</sup>H- and <sup>13</sup>C NMR: Tables 1 and 2, respectively.

**Compound 5:** Colorless needles, mp 212° (MeOH). IR  $\nu^{KBr}$  cm<sup>-1</sup>: 3300-3600, 1660, 1610, 1500, 1460 and 1370. UV  $\lambda_{max}$  nm, MeOH: 225, 290, 335(sh); +NaOMe: 250, 326; MeOH + AlCl<sub>3</sub>: 310, 372; MeOH + AlCl<sub>3</sub>+HCl: 309, 375; MeOH + NaOAc: 285(sh), 329; MeOH + NaOAc+H<sub>3</sub>BO<sub>3</sub>: 290, 329(sh). EI MS (direct inlet) m/z (rel. int. %): 434[M]<sup>+</sup> (0.0), 416[M-1H<sub>2</sub>O]<sup>+</sup> (38), 398 [M-2H<sub>2</sub>O]<sup>+</sup> (7), 380[M-3H<sub>2</sub>O]<sup>+</sup> (4), 286[M-148]<sup>+</sup> (13), 285[M-149]<sup>+</sup> (33), 272(36), 271(20), 165(99), 153(29), 152(19), 120(100), 69(28) and 55(25). FAB MS: 435[M+1]<sup>+</sup>, 391 and 287. <sup>1</sup>H- and <sup>13</sup>C NMR: Tables 1 and 2, respectively.

**Compound 6:** Yellow microneedles, mp 182° (MeOH). IR  $\nu^{KBr}$  cm<sup>-1</sup>: 3200-3500, 1660, 1610, 1575, 1500, 1460 and 1370. UV  $\lambda_{max}$  nm, MeOH: 255, 262(sh), 300(sh), 352; +NaOMe: 269, 328, 397; MeOH + AlCl<sub>3</sub>: 272, 305(sh), 328, 429; MeOH + AlCl<sub>3</sub>+HCl: 269, 300(sh), 354, 399; MeOH + NaOAc: 267, 320(sh), 360; MeOH + NaOAc+H<sub>3</sub>BO<sub>3</sub>: 259, 300(sh), 370. EI MS m/z (rel. int. %): 302[M-rham.]<sup>+</sup> (100), 301(15), 273(7), 153(9), 137(13) and 128(13). FAB MS: 449[M-1]<sup>+</sup> and 303[aglycone+1]<sup>+</sup>. <sup>1</sup>H- and <sup>13</sup>C NMR: Tables 1 and 2, respectively.

$\beta$ -sitosterol and  $\beta$ -sitosterol-O-glucoside were identified by direct comparison (mp, mmp, co-TLC, IR and MS).

**Hydrolysis of 3, 4 and 6<sup>(39)</sup>:** A suspension of the compound (50 mg) in H<sub>2</sub>O-conc. HCl 9:1 (5 ml) was heated under reflux for 4hr (or until hydrolysis was complete as indicated by TLC monitoring), cooled and extracted with EtOAc. The presence of sugar was demonstrated in the aq. solutions by direct co-PC (n-BuOH-HOAc-H<sub>2</sub>O, 4:1:5, upper phase), and co-TLC (silica gel n-BuOH-HOAc-Et<sub>2</sub>O-H<sub>2</sub>O, 9:6:3:1) with authentic sample of glucose (in case of 3 and 4) and rhamnose (in case of 6). The EtOAc extract contained the aglycone which was identified as naringenin (in case of 3 and 4) and quercetin (in case of 6), by direct comparison (co-TLC, IR, UV, MS and <sup>1</sup>H NMR) with 1 (naringenin) and 2 (quercetin), respectively. Compound 5 was subjected to the same hydrolysis procedure but neither sugar nor aglycone could be detected<sup>(46)</sup>.

## RESULTS AND DISCUSSION

The alcoholic extract of *A. saligna* flowers was partitioned between water and ether then ethyl acetate. Repeated chromatographic separations of the ether portion provided  $\beta$ -sitosterol,  $\beta$ -sitosterol glucoside and compound 1, while the ethyl acetate provided compounds 2-6.

Compound 1 showed UV absorptions (330sh and 289 nm) and  $^1\text{H}$  NMR data (Table 1) typical for flavanones<sup>(35,36)</sup>. The later showed three pairs of doublets at  $\delta$  5.41, 2.67 and 3.23, typical for H-1, H-3<sub>eq</sub> and H-3<sub>ax</sub> of a flavanone<sup>(36)</sup>; A<sub>2</sub>B<sub>2</sub> system ( $\delta$  7.30,d and 6.79,d) characteristic for 4'-substituted ring B, and a singlet at  $\delta$  5.88 integrated for 2 protons, assignable to H-8 and H-6<sup>(36)</sup>. The presence of 5-OH group could be concluded from the highly deshielded OH singlet at  $\delta$  12.14 and also by the band II shift (+20 nm) in the UV spectrum when  $\text{AlCl}_3 + \text{HCl}$  were added.<sup>(35)</sup> These data suggested a 5,7,4'-trihydroxy flavanone. The EI MS spectrum showed a molecular ion peak [M]<sup>+</sup> at m/z 272 corresponding to the molecular formula C<sub>15</sub>H<sub>12</sub>O<sub>5</sub> and other fragments typical for naringenin<sup>(37-39)</sup>. Comparison of the physical and spectral data (mp, IR, MS, and  $^1\text{H}$ - and  $^{13}\text{C}$  NMR) of 1 with those reported for naringenin<sup>(36-42)</sup> provided firm evidence that 1 is naringenin. This compound has been isolated from *A. longifolia* before.<sup>(43)</sup>

Compound 4 showed similar UV spectra to that of 1. Its  $^1\text{H}$ - and  $^{13}\text{C}$  NMR data revealed 7 sugar protons and 6 carbons signals, respectively, more than those of 1, indicating its glycosidic nature. The EI MS showed an intense peak at m/z 272 [naringenin]<sup>+</sup> and a fragmentation pattern similar to that of 1. The FAB MS revealed a molecular ion at m/z 435 [M+1]<sup>+</sup> identical with the molecular formula C<sub>21</sub>H<sub>22</sub>O<sub>16</sub>. These data suggested the presence of naringenin hexoside. The  $^1\text{H}$  NMR spectrum of 4 showed two separate singlets for H-6 ( $\delta$  6.32,d) and H-8 ( $\delta$  6.06,d), instead of two protons singlet in 1. It also revealed a doublet at  $\delta$  4.76,d (J=7 Hz) assigned for H-1'' of a  $\beta$ -glucosyl moiety.<sup>(36)</sup> The  $^{13}\text{C}$  NMR spectra revealed a methylene carbon at  $\delta$  60.74,t (C-6'') and an anomeric carbon resonance at  $\delta$  102.11,s. These data suggested that 4 is naringenin 7-O- $\beta$ -glucoside. This conclusion was confirmed by comparing these data with those reported for naringenin 7-O- $\beta$ -glucoside<sup>(42,44)</sup> and similar compounds<sup>(35,36,39,40,41,45)</sup>. Acid hydrolysis of 4 gave glucose and naringenin (see Experimental).

Compound 3 was obtained as colorless crystals, mp 218°. It showed a higher R<sub>f</sub> value compared to 4 and its IR spectrum showed an additional absorption band at 1735 cm<sup>-1</sup> (C=O of acetate). The EI MS showed an intense peak at m/z 272 [naringenin]<sup>+</sup> and a fragmentation pattern similar to that of 1 and 4. The  $^1\text{H}$ -NMR spectrum of 3 was very similar to that of 4

except for the appearance of a three protons singlet at  $\delta$  2.03 assignable to an acetate methyl<sup>(45)</sup>, the anomeric proton H-1'' was slightly shifted downfield ( $\delta$  4.83, J=6.9 Hz) and one of the two mutually coupled H-6'' protons signals was observed at  $\delta$  3.88,dd (in 4 the later signals were not distinguishable being hidden under the other sugar resonances). The  $^{13}\text{C}$  NMR spectrum was also very similar to that of 4, but two additional resonances were observed at  $\delta$  173.50,s and 20.75,q, assignable to a quaternary (C=O) and a methyl carbon of acetoxy group.<sup>(45)</sup> In addition, the glucose C-6'' signal was shifted 4.55 ppm downfield, while C-5'' signal was shifted 1.64 ppm upfield, suggested esterification at C-6''.<sup>(45)</sup> These NMR data suggested that 3 is an ester of 4 and the acid involved is acetic acid<sup>(45)</sup>. The point of attachment was established by examining the  $^1\text{H}$ - and  $^{13}\text{C}$  NMR resonances in comparison to those of 4 (as discussed above). Thus, it was concluded that 3 is naringenin-7- $\beta$ -D-glucoside 6''-acetate. This was supported by comparing these data with those published for quite similar compounds<sup>(39,45)</sup>. Acid hydrolysis furnished naringenin and glucose (as the acetyl radical was removed during hydrolysis)<sup>(45)</sup>. Assignments were confirmed by APT, DEPT,  $^1\text{H}$ - $^{13}\text{C}$  HETCOR experiments. These data provided evidence that 3 is naringenin-6-- $\beta$ -D-glucoside 6''-acetate. The available literature indicated that 3 is a new compound, that has not been isolated from natural sources before. However, naringin 6''-acetate was previously prepared by decarboxylation of naringenin 6''-malonate obtained from *Citrus paradisi*<sup>(45)</sup>.

Compound 5 showed UV spectrum close to that of 1. Its lower R<sub>f</sub> value in comparison to 1 besides its resistance to acid hydrolysis (neither sugar nor aglycone were obtained), suggested a C-glycoside.<sup>(36,46)</sup> The  $^1\text{H}$  NMR spectrum showed signals similar to those of 1 in addition to those attributable to a hexosyle moiety at  $\delta$  3.10-3.50,m and its anomeric proton at  $\delta$  4.49,d. The H-6 and H-8 protons which appeared as a singlet at  $\delta$  5.88 in 1, and as two doublets at  $\delta$  6.32 and 6.06 in 4 are replaced by a singlet integrated for only one proton (H-8) at  $\delta$  5.93. In view of these results 5 is shown to be a C-hexoside of a flavanone with the C-sugar in either the 6- or 8-position. The  $^{13}\text{C}$  NMR spectra showed signals due to the glucosyl moiety as in 4, however, the anomeric carbon C-1'' was further upfield at  $\delta$  73.01 (this carbon usually resonates around  $\delta$  100 in O-glucosides)<sup>(40,41)</sup>, and C-6 appeared as a singlet at  $\delta$  105.83. This indicated a C-6 glucoside. The MS data (see Experimental) of the underivatized compound 5 showed that the molecular ion peak was not observed, however it showed three peaks due to the sequential loss of three molecules of water<sup>(46)</sup>. The intensity of [M-148]<sup>+</sup> peak relative to the [M-149]<sup>+</sup> peak was 40%, thus indicating that the sugar moiety must be attached to C-6 position of the flavonoid.<sup>(46)</sup>

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## مواد فلافونوية من أزهار نبات أكاشيا ساليجنا

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تم في هذا البحث فصل ستة مواد فلافونوية منها مادة جديدة وأخرى لم تفصل من نباتات جنس الأكاشيا من قبل. هذا بالإضافة إلى مادتي بيتاستيستيرون وبيتاستيستيرون جلوكوزيد. وقد أمكن التعرف على التركيب البنائي لهذه المركبات من خلال دراسة خواصها الطبيعية وكذا دراسة أطباق الأشعة تحت الحمراء وفوق البنفسجية وطيف الكتلة والطينين النووي المغناطيسي للهيدروجين-1 والكربون-13 ذو البعد الواحد وذو البعدين. وأظهرت النتائج أن هذه المواد الفلافونوية هي: نارينجينين ونارينجينين-7-أو-بيتا-دي-جلوكوزيد-6-أستيات (وهو مركب جديد) ونارينجينين-7-أو-بيتا-دي-جلوكوزيد (وهو مركب تم فصله هنا لأول مرة من جنس أكاشيا) و6-ك-جلوكوزيل نارينجينين و كورستين وكورستينين. هذا وقد تم مناقشة أهمية نتائج هذه البحث من منظور التصنيف الكيميائي لنباتات جنس الأكاشيا وكذا استخدام هذه المواد المفصلة كدلائل لتمييز نبات أكاشيا ساليجنا عن نباتات نفس الجنس المشابه.