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ISOFLAVONOIDS AND CHALCONES FROM *ANTHYLLIS HERMANNIAE*

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Key Word Index—*Anthyllis hermanniae*; Fabaceae; prenylated isoflavonoids; chalcones; anthyllisone; anthyllin.

Abstract—From the aerial parts of *Anthyllis hermanniae* two new prenylated chalcones, 1-(2,4-dihydroxyphenyl)-3-[2,2-dimethyl-8-(3-methyl-2-butenyl)-2*H*-1-benzopyran-6-yl]-2-propen-1-one (anthyllisone) and 1-(2,4-dihydroxyphenyl)-3-[4-hydroxy-3-(2-hydroxy-3-methyl-3-butenyl)-5-(3-methyl-2-butenyl)phenyl]-2-propen-1-one (anthyllin), have been isolated, along with the known chalcones isobavachalcone and abyssinone VI and the prenylated isoflavonoids wighteone, lupiwighteone and lupalbigenin. Their structures were elucidated by spectroscopic methods, including 2D NMR techniques. D-Pinitol was also isolated.

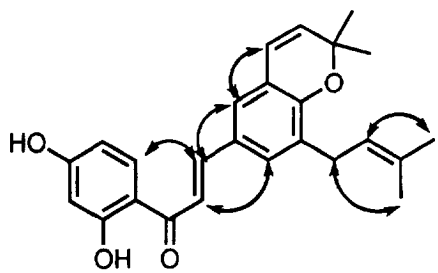
INTRODUCTION

Anthyllis hermanniae L. is a shrub with tortuous, woody branches, typical of the Mediterranean region [1]. Most papers on the genus *Anthyllis* are related to the flavonoid content [2]. We report here the isolation and the structural elucidation of two new isoprenoid chalcones: anthyllisone (**1**) and anthyllin (**2**), along with the known chalcones isobavachalcone (**3**) and abyssinone VI (**4**) and the prenylated isoflavonoids wighteone (**5**), lupiwighteone (**6**) and lupalbigenin (**7**).

RESULTS AND DISCUSSION

Compound **1** was isolated from the *n*-hexane extract and gave a brown spot on a TLC plate with the methanolic ferric chloride test, while treatment with ceric sulphate in sulphuric acid gave an orange-reddish colour. Under UV light (λ 366 nm) the yellow spot on TLC changed to a yellow-green fluorescence. In the IR spectrum the absorption bands due to the phenolic hydroxyls (3350 cm^{-1}) and carbonyl function (1640 cm^{-1}) were discernible. Its UV spectral data (λ_{max} 380.9 and 284.5 nm) and the bathochromic shifts induced by $AlCl_3$–HCl and sodium methoxide suggested the presence of 2′,4′-dihydroxychalcone [3].

Two doublets at δ 7.75 and 7.61 (J = 15.3 Hz) in the protonic spectrum (methanol-d_4) were assigned to the AB system of a chalcone, and the singlet at δ 13.6 to one chelated hydroxyl group. Moreover, the ^{1}H NMR spectrum showed signals for a prenyl group [3,3-dimethylallyl group: δ 1.74 and 1.77 (each 3H, *s*), 3.25 (2H, *m*) and 5.27 (1H, *t*-like *m*)], for AXY-type aromatic protons (A ring): δ 7.98 (1H, *d*, J = 8.8 Hz), 6.42 (1H, *dd*, J = 8.8 and 2.2) and 6.29 (1H, *d*, J = 2.2 Hz) and two aromatic broad singlets (δ 7.32 and 7.35) for *meta*-coupled B-ring protons. The spin network of the AXY system was confirmed by a ^{1}H–^{1}H COSY spectrum. The presence of a 2,2-dimethylpyran ring was deduced from the 6H singlet at δ 1.43 due to the *gem*-dimethyl group and from the two *ortho*-cou-



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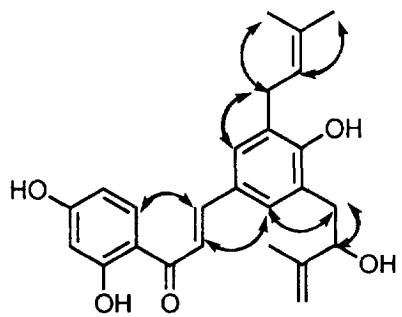

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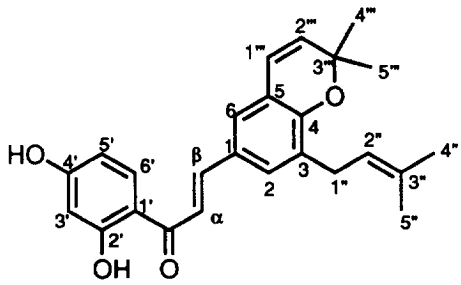
Fig. 1. 2D NOESY of **1** and **2**.



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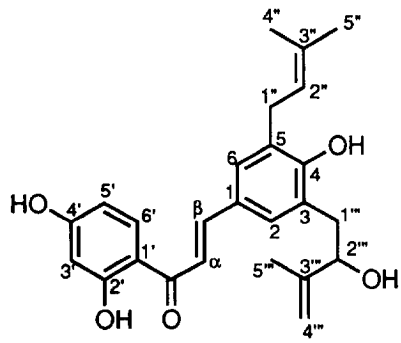

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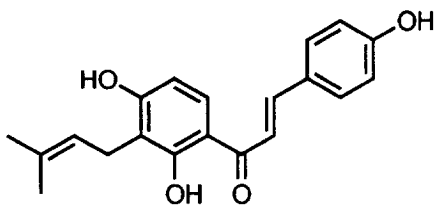

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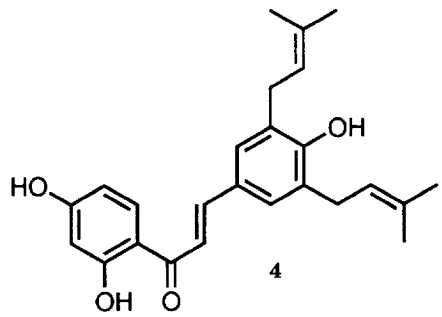

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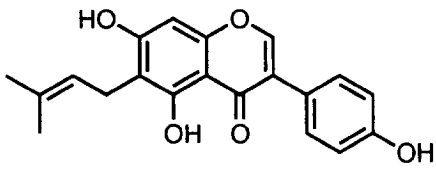

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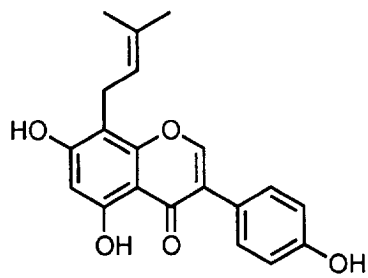

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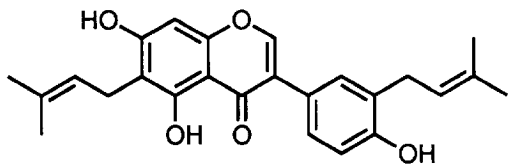

7

pled aromatic protons at δ 5.75 (*d*, $J = 9.8$ Hz) and 6.41 (*d*, $J = 9.8$ Hz) assignable to H-2‴ and H-1‴, respectively.

The EI mass spectrum of **1** furnished a $[M]^+$ at m/z 390 according with the molecular formula $C_{25}H_{26}O_4$, confirmed also by ^{13}C NMR and DEPT spectra. The mass spectrum showed characteristic fragmentation ions at m/z 375 for $[M - Me]^+$, typical of compounds with a 2,2-dimethylpyran system, and at m/z 137, which suggested that the chromene group was located on the B ring. These data indicated that both the 3,3-dimethylallyl group and the 2,2-dimethylchromene moiety must be placed on the B ring and this suggests four possible substitution patterns, for this ring. The chosen isomer **1** is to be preferred considering its co-existence with **4** and by the analysis of the NOESY spectrum, as described in Fig. 1. Finally, the ^{13}C NMR data supported the proposed structure, by comparison with values for model compounds [4]. From these spectral data, **1**, which we name anthyllisone, was

Table 1. ^{13}C NMR data for compounds **1**–**2*** (in CD_3OD)

C	1	2	C	1	2
C=O	193.5	193.6	5′	109.2	109.2
α	118.9	118.2	6′	133.4	133.3
β	145.6	146.1	1″	29.3	29.6
1	131.0	131.0	2″	123.6	123.7
2	125.7	130.4	3″	129.8	129.8
3	122.7	128.2†	4″	18.0	17.9
4	154.6	158.2	5″	25.9	25.9
5	128.7	127.8†	1‴	123.1	39.5
6	132.3	130.4	2‴	131.7	77.7
1′	114.7	114.7	3‴	78.3	148.5
2′	166.4	166.5	4‴	28.4	111.0
3′	103.8	103.9	5‴	28.4	18.3
4′	167.5	167.5			

*The number of directly attached protons were verified with DEPT pulse sequence.

†Values may be reversed in the same column.

identified as 1-(2,4-dihydroxyphenyl)-3-[2,2-dimethyl-8-(3-methyl-2-butenyl)-2*H*-1-benzopyran-6-yl]-2-propen-1-one.

Compound **2**, isolated from the chloroform extract, gave a brown colour with the methanolic ferric chloride test and a red one with H_2SO_4–methanol. The IR bands at 1630 and 3400 cm^{-1} can be attributed to chelated carbonyl and phenolic hydroxyl groups, respectively. The UV spectrum resembled that of a chalcone (λ_{max} 376 and 262 nm). The EI mass spectrum gave signals corresponding to a compound with a molecular formula $C_{25}H_{28}O_5$, also confirmed by ^{13}C NMR and DEPT spectra. The ^{13}C NMR spectrum showed 25 signals that were attributed, on the basis of DEPT experiments, to: Me × 3, CH_2 × 3, =CH × 9, =C × 9 and C=O × 1.

The ^1H NMR spectrum (methanol-d_4) indicated that the compound was a 2′,4′-dihydroxychalcone derivative [δ 7.57 (*d*, J = 15.4 Hz, H-α), 7.75 (*d*, J = 15.4 Hz, H-β), 13.77 (*s*, OH-2′), 7.97 (*d*, J = 8.8 Hz, H-6′), 6.43 (*dd*, J = 8.8 and 2.4 Hz, H-5′) and 6.39 (*d*, J = 2.4 Hz, H-3′) for an AXY system]. Other signals, i.e. the signals of a prenyl chain and two *meta*-coupled aromatic protons (B ring) appeared similar to those of **1**.

The ^1H NMR spectrum of **2** was closely related to that of abyssinone VI, except for the presence of signals assignable to a 2-hydroxy-3-methyl-3-butenyl moiety [δ 1.82 (3H, *s*, H-5‴), 2.91 (2H, *m*, J = 7.0 and 3.8 Hz, H-1‴), 4.35 (1H, *dd*, H-2‴), 4.83 and 4.99 (each 1H, *br s*, H-4‴)] instead of the second 3,3-dimethylallyl chain. The mass spectrum showed characteristic fragment ions at *m/z* 137 (2′,4′-dihydroxyl A ring), 282 and 239 (B ring). The complete structural elucidation was established on the basis of the chemical shifts and J values of the ^1H NMR spectrum and from detailed spectral analyses of ^{13}C NMR, COSY and HETCOR. From the above data, it was suggested that the two chains were on the B ring, also confirmed by NOESY experiments, as evidenced in Fig. 1. Thus, the structure of anthyllin (**2**) is 1-(2,4-dihydroxyphenyl)-3-[4-hydroxy-3-(2-hydroxy-3-methyl-3-butenyl)-5-(3-methyl-2-butenyl)phenyl]-2-propen-1-one.

The known compounds isobavachalcone (**3**) [5], abyssinone VI (**4**) [6, 7], wighteone (**5**) [8, 9], lupiwighteone (**6**) [10, 11] and lupalbigenin (**7**) [12] were also isolated and identified by comparison of their spectral and physical data with those reported in the literature. The ^{13}C spectral data for **3** and **7** are reported here for the first time. Assignments for ^{13}C were made on the basis of COSY and HETCOR experiments as well as the multiplicities observed in DEPT spectra. Isoflavonoids and chalcones are rather unusual in the tribe Loteae, to which the genus *Anthyllis* belongs, although they are very common constituents in many tribes of Fabaceae [13]. The presence of a great amount of D-pinitol could represent the response of this plant to its arid environment [14].

EXPERIMENTAL

General. UV spectra were recorded in MeOH; IR spectra as nujol mulls. ^1H and ^{13}C NMR were recorded at 200 and 50 MHz, respectively, in MeOH-d_4 and $(Me)_2CO$-d_6 (TMS as int. standard). Carbon multiplicities were determined by DEPT 90° and 135° pulse sequence. COSY, HETCOR and NOESY were performed using standard Bruker software. EI-MS: direct inlet, HP 5988 A (70 eV). TLC was carried out on silica gel and RP-8 plates (Merck). Lobar RP-8 (40–63 μm, Merck), Sephadex LH-20 (Pharmacia) and silica gel 60 (230–400 mesh, Merck) were used for CC.

Plant material. Anthyllis hermanniae was collected in June 1993 in Sardinia (Monte Tolui), where it grows in an arid environment. A voucher specimen is deposited at the Dipartimento di Chimica Bioorganica, Pisa.

Extraction and isolation. Air-dried powdered aerial parts of *A. hermanniae* (700 g) were extracted in a Soxhlet with *n*-hexane, $CHCl_3$ and $CHCl_3$–MeOH (9:1). The residue (11.8 g) obtained by evapn of the *n*-hexane extract was partitioned between *n*-hexane and 90% aq. MeOH. A part of the MeOH layer (1.0 g) was subjected to flash chromatography (*n*-hexane–EtOAc, 3:2) and subsequently to CC on Sephadex LH-20 with MeOH to give A–H frs. Fr. D (60 mg) was fractionated by LPLC on a Lobar RP-8 column, eluting with MeOH–H_2O (17:3), yielding **4** (34 mg) and **1** (13 mg). From fr. E (72 mg) was obtained **7** (12 mg) by low pressure Lobar RP8 CC (MeOH–H_2O, 17:3) and final purification by prep. TLC (*n*-hexane–EtOAc, 3:2).

The $CHCl_3$ extract (9.4 g) was subjected to CC on Sephadex LH-20 using MeOH–$CHCl_3$ (9:1) as mobile phase. The frs were monitored by TLC on Si gel 60 using $CHCl_3$–MeOH (4:1) or $CHCl_3$–Et_2O (7:3) as eluents and detected by $Ce(SO_4)_2/H_2SO_4$ as spray reagent. The frs were further purified by Lobar RP8 (eluent MeOH–H_2O, 4:1) to yield **5** (15.4 mg), **6** (15.5 mg), **7** (50.5 mg), **4** (78.5 mg), **3** (20 mg) and **2** (7.5 mg).

Known compounds were characterized by compari-

son of their physical and spectral properties with those of authentic samples and with those reported in refs. [5–12].

The $CHCl_3$–MeOH extract, after evapn of the solvent, gave a ppt. (1.3 g) identified as D-pinitol by comparison of its mp and $[\alpha]_D^{20}$ with those in Ref. [15] and by co-TLC with an authentic sample.

Anthyllisone (**1**). TLC: RP8, MeOH–H_2O (17:3): R_f 0.4; IR ν_{max}^{nujol} cm^{-1} 3350, 1640, 1530, 1380; UV λ_{max}^{MeOH} nm: 380.9, 284.5 (sh), 255.6 (sh); $\lambda_{max}^{MeOH+AlCl_3}$ 440, 360 (sh), 310 (sh); EIMS *m/z* (rel. int. %): 390 $[M]^+$ (3.37), 375 $[M-15]^+$ (26.11), 165 (4.48), 137 (19.54), 97 (23.18), 57 (4.056), 55 (35.35), 43 (100), 18 (90); ^{1}H NMR (MeOH-d_4): δ 13.6 (*s*, OH-2′), 7.98 (1H, *d*, J = 8.8 Hz, H-6′), 7.75 (1H, *d*, J = 15.3 Hz, H-β), 7.61 (1H, *d*, J = 15.3 Hz, H-α), 7.35 (1H, *br s*, H-2), 7.32 (1H, *br s*, H-6), 6.42 (1H, *dd*, J = 8.8, 2.2 Hz, H-5′), 6.41 (1H, *d*, J = 8.9 Hz, H-1‴), 6.29 (1H, *d*, J = 2.2 Hz, H-3′), 5.75 (1H, *d*, J = 8.9 Hz, H-2‴), 5.27 (1H, *t*-like *m*, H-2″), 3.25 (2H, *m*, H-1″), 1.77 (3H, *br s*, H-5″), 1.74 (3H, *br s*, H-4″), 1.43 (6H, *s*, H-4‴/5‴); ^{13}C NMR (MeOH-d_4): see Table 1.

Anthyllin (**2**). TLC: silica gel 60, $CHCl_3$–Et_2O (7:3) R_f 0.4; IR ν_{max}^{nujol} cm^{-1} 3400, 1630, 1565; UV λ_{max}^{MeOH} nm: 376, 262 (sh); $\lambda_{max}^{MeOH+AlCl_3}$ 428.6, 320 (sh), 270 (sh); $\lambda_{max}^{MeOH+NaOMe}$ 445, 340 (sh), 280 (sH), 256 (sh); EIMS *m/z* (rel. int. %): 408 $[M]^+$ (5.24), 339 (25.80), 282 (7.10), 241 (10.17), 239 (19.19), 185 (21.53), 163 (20.27), 137 (100), 81 (17.92), 55 (21.90), 69 (35.23), 43 (40.14); ^{1}H NMR (MeOH-d_4): δ 13.77 (*s*, OH-2′), 7.97 (1H, *d*, J = 8.8 Hz, H-6′), 7.75 (1H, *d*, J = 15.4 Hz, H-β), 7.57 (1H, *d*, J = 15.4 Hz, H-α), 7.39 (1H, *d*, J = 2.0 Hz, H-2), 7.33 (1H, *d*, J = 2.0 Hz, H-6), 6.43 (1H, *dd*, J = 8.8, 2.4 Hz, H-5′), 6.39 (1H, *d*, J = 2.4 Hz, H-3′), 5.34 (1H, *t*-like *m*, H-2″), 4.99, 4.83 (2H, *br s*, H-4‴), 4.35 (1H, *dd*, J = 7.0, 3.8 Hz, H-2‴), 3.35 (2H, *m*, H-1″), 2.91 (2H, *m*, H-1‴), 1.82 (3H, *s*, H-5‴), 1.75 (6H, *br s*, H-4″, H-5″); ^{13}C NMR (MeOH-d_4): see Table 1.

Isobavachalcone (**3**). IR ν_{max}^{nujol} cm^{-1} 3400, 1630, 1565; UV λ_{max}^{MeOH} nm: 367.8; $\lambda_{max}^{MeOH+AlCl_3}$ 376, 320 (sh); $\lambda_{max}^{MeOH+NaOMe}$ 434, 348 (sh); EIMS *m/z* (rel. int. %): 324 $[M]^+$ (36.10), 281 (82.34), 269 (21.25), 203 (15.31), 161 (56.8), 149 (100), 147 (39), 120 (27.96), 55 (12.51), 43 (14.69), 18 (19.56); ^{1}H NMR (MeOH-d_4): δ 7.83 (1H, *d*, J = 8.9 Hz, H-6′), 7.78 (1H, *d*, J = 15.4 Hz, H-β), 7.61 (1H, *d*, J = 15.2 Hz, H-α), 7.60 (2H, *d*, J = 8.6 Hz, H-2, H-6), 6.84 (1H, *d*, J = 8.6 Hz, H-3, H-5), 6.43 (1H, *d*, J = 8.9 Hz, H-5′), 5.23 (2H, *t*-like *m*, H-2″), 3.35 (2H, *m*, H-1″), 1.66 (3H, *br s*, H-4″), 1.78 (3H, *br s*, H-5″); ^{13}C NMR (MeOH-d_4): δ 193.7 (C=O), 165.0 (C-4′), 163.7 (C-2′), 161.4 (C-4), 145.3 (C-β), 131.9 (C-3″), 131.7 (C-2, C-6), 130.4 (C-6′), 127.9 (C-1), 123.6 (C-2″), 118.6 (C-α), 117.0 (C-3, C-5), 116.6 (C-3′), 114.5 (C-1′), 108.3 (C-5′), 25.9 (C-5″), 22.5 (C-1″), 17.9 (C-4″).

Lupalbigenin (**7**). IR ν_{max}^{nujol} cm^{-1} 3380, 1640, 1620; UV λ_{max}^{MeOH} nm: 267.8, 348 (sh); $\lambda_{max}^{MeOH+AlCl_3}$ 281.2, 322 (sh), 375 (sh); $\lambda_{max}^{MeOH+NaOMe}$ 337, 275.6 (sh); EIMS *m/z* (rel. int. %): 406 (45.6), 363 (67.81), 351 (100), 307 (51.9), 295 (48.43), 69 (35.35), 55 (18.6), 43 (16.2), 41 (19.7); ^{1}H-NMR (Me_2CO-d_6) δ 13.17 (1H, *s*, OH-2), 8.1 (1H, *s*, H-2), 7.33 (1H, *d*, J = 2.1 Hz, H-2′), 7.27 (1H, *dd*, J = 8.2, 2.3 Hz, H-6′), 6.88 (1H, *d*, J = 8.2 Hz, H-5′), 6.48 (1H, *s*, H-8), 5.37 (1H, *t*-like *m*, J = 7.2 Hz, H-2‴), 5.28 (1H, *t*-like *m*, J = 7.2 Hz, H-2″), 3.36 (4H, *d*, J = 7.2 Hz, H-1‴, H-1″), 1.74 (3H, *br s*, H-5″), 1.73 (3H, *br s*, H-5‴), 1.71 (3H, *br s*, H-4‴), 1.65 (3H, *br s*, H-4″); ^{13}C NMR (Me_2CO-d_6): δ 181.7 (C-4), 162.5 (C-7), 160.5 (C-5), 156.7 (C-4′), 155.8 (C-9), 153.9 (C-2), 131.6 (C-3″), 131.5 (C-3‴), 131.2 (C-2′), 128.5 (C-6′), 124.1 (C-3′), 123.6 (C-2‴), 123.3 (C-1′), 123.2 (C-3), 123.1 (C-2″), 115.5 (C-5′), 112.3 (C-6), 106 (C-10), 93.7 (C-8), 29.1 (C-1″), 25.8 (C-4″), 25.8 (C-4‴), 22.0 (C-1‴), 17.8 (C-5″), 17.6 (C-5‴).

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