

PHENYLPROPANOIDS FROM *BUPLEURUM FRUTICOSUM*LUISA PISTELLI,* ANNA RITA BILIA, ALESSANDRA BERTOLI,
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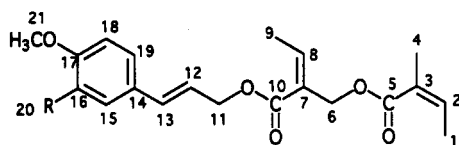
ABSTRACT.—Two new phenylpropanoids, (*E*)-3-(3,4-dimethoxyphenyl)-2-propen-1-yl (*Z*)-2-[(*Z*)-2-methyl-2-butenyloxymethyl]butenoate [**1**], and (*E*)-3-(4-methoxyphenyl)-2-propen-1-yl (*Z*)-2-[(*Z*)-2-methyl-2-butenyloxymethyl]butenoate [**2**], were isolated from the *n*-hexane extract of the leaves of *Bupleurum fruticosum*. Their structures were established by spectral methods.

We have reported previously on the isolation of three saponins from the roots of *Bupleurum fruticosum* L. (Apiaceae) (1). The structure elucidation of two novel phenylpropanoids [**1** and **2**], obtained from an *n*-hexane extract of the leaves of this species, is presented herein. 1,2-Dimethoxy-4-(1-methoxy-2-propenyl) benzene [**3**], 1,2-dimethoxy-4-(3-methoxy-1-propenyl)benzene [**4**], 3,4-

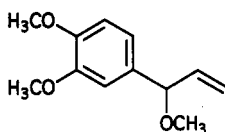
dimethoxycinnamaldehyde [**5**], and 7-(3,3-dimethylallyloxy)-6-hydroxycoumarin (prenyletin, [**6**]), were also found in the same extract.

The residue obtained on evaporation of an *n*-hexane extract of air-dried leaves of *Bupleurum fruticosum* was partitioned between *n*-hexane and 90% aqueous MeOH. The MeOH layer was subjected to Sephadex LH-20 gel filtration and was subsequently flash chromatographed to yield compounds **1** to **6**.

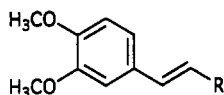
The ir spectrum of compound **1** showed bands attributable to α,β -unsaturated ester ($1718, 1640\text{ cm}^{-1}$) and aromatic ether (1265 cm^{-1}) absorption. The molecular formula of $\text{C}_{21}\text{H}_{26}\text{O}_6$ was deduced from both elemental analysis and eims (molecular peak at m/z 374). The ^{13}C -nmr spectrum of **1** revealed twenty-one signals; these were sorted, by DEPT experiments, into $\text{Me}\times 3$, $\text{MeO}\times 2$, $\text{OCH}_2\times 2$, $=\text{CH}\times 7$, $=\text{C}\times 5$, and $\text{COO}\times 2$ (Table 1). The ^1H -nmr spectrum (Table 2) showed signals attributable to two aromatic methoxyl groups at δ 3.88 and 3.90. In addition, the aromatic resonances were typical of an unsymmetrically trisubstituted benzene ring, as evidenced by three signals located at δ 6.81 (d, $J=8.5$ Hz), 6.92 (dd, $J=8.5$ and 2.3 Hz), and 6.94 (d, $J=2.3$ Hz). A careful examination of the multiplicity of the other ^1H -nmr signals and their coupling constants as well as 2D ^1H - ^1H nmr experiments (Table 2) indicated three isolated spin-systems of the non-aromatic moiety of **1**: one (fragment



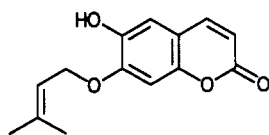
*1 R = OCH₃
2 R = H



3



4 R = CH₂OCH₃
5 R = CHO



6

*The numbering of carbon atoms in structures **1** and **2** is not according to IUPAC rules.

TABLE 1. ^{13}C -Nmr Data of Compounds **1** and **2** (CDCl_3 , 200 MHz).

Carbon	Compound					
	1			2		
	^{13}C	DEPT	HETCOR	^{13}C	DEPT	HETCOR
1	16.5	CH_3	1.94	16.5	CH_3	1.88
2	138.7	CH	6.03	138.8	CH	6.04
3	128.3	C		129.0	C	
4	21.2	CH_3	1.85	21.2	CH_3	1.84
5	160.4 ^a	CO		166.5 ^a	CO	
6	67.7	CH_2	4.84	65.9	CH_2	4.76
7	128.4	C		128.5	C	
8	144.1	CH	6.48	144.6	CH	6.41
9	16.5	CH_3	2.12	16.5	CH_3	2.05
10	168.2 ^a	CO		168.0 ^a	CO	
11	67.7	CH_2	4.82	65.9	CH_2	4.74
12	121.6	CH	6.18	121.4	CH	6.15
13	143.8	CH	6.61	144.1	CH	6.54
14	129.9	C		129.6	C	
15	109.4	CH	6.94	128.5	CH	7.24
16	149.8	C		114.6	CH	6.78
17	149.6	C		154.7	C	
18	111.6	CH	6.81	114.6	CH	6.78
19	120.6	CH	6.92	128.5	CH	7.24
20	56.5	CH_3	3.88			
21	56.5	CH_3	3.90	56.0	CH_3	3.74

^aThese assignments may be interchanged in the same column.

A in the formula) consisting of two vinylic CH_3 groups (δ 1.85 and 1.94) and an olefinic H (δ 6.03); another (fragment B) formed by a vinylic CH_3 group (δ 2.12), a vinylic CH_2O group (δ 4.84), and an olefinic H (δ 6.48); a third (fragment C) containing two trans-coupled olefinic H (δ 6.18 and 6.61) atoms and a CH_2O group (δ 4.82) (Figure 1). HETCOR nmr experiments correlated the carbon signals with their associated proton signals as summarized in Table 1, while COLOC nmr experiments established the resonances of the quaternary carbons (Figure 2).

Thus, from the three-bond couplings, the proton resonances of the two methoxyl groups at δ 3.88 and 3.90 showed correlations with the ^{13}C -nmr signals at 149.8 and 149.6 ppm, respectively. Furthermore, the ^{13}C -nmr signal at 149.8 ppm showed a cross-peak correlation with the proton resonance at δ 6.94 (H-2 of the aromatic system). Therefore, methoxyls

at C-3 and C-4 of the phenyl moiety (C-16 and C-17) were unequivocally established. Moreover, the resonance at δ 6.61, due to one olefinic proton in fragment C, correlated with the carbon resonance at 129.9 ppm (C-1 of the aromatic system) and the presence of a 3,4-dimethoxycinnamyl moiety was indicated. Finally, the ^{13}C -nmr resonances of the quaternary vinylic carbons of fragments A and B were established by the cross-peak correlations between the ^1H -nmr resonance at δ 1.85 and the ^{13}C -nmr resonance at 128.3 ppm, and between the ^1H -nmr resonance at δ 4.84 and the ^{13}C -nmr resonance at 128.4 ppm, respectively.

The sequence of the three fragments was finally deduced by eims (Figure 1): the peak at m/z 275 [$\text{M}-99$]⁺ was due to the loss of fragment A, while the peak at m/z 193 [$\text{M}-(99+82)$]⁺ arose from the loss of fragments A and B. Thus, the structure of **1** corresponded to (*E*)-3-(3,4-dimethoxyphenyl)-2-propen-1-yl

TABLE 2. $^1\text{H-Nmr}$ Data of Compounds **1** and **2** (CDCl_3 , 200 MHz).

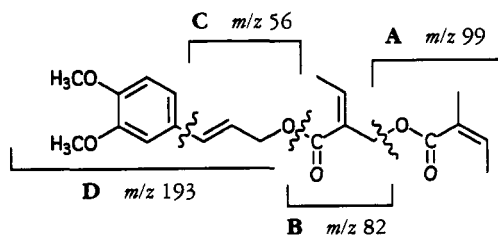
Proton	Compound			
	1		2	
	$^1\text{H nmr}$	COSY	$^1\text{H nmr}$	COSY
1	1.94 (3H, dq, $J=7.2, 1.6$ Hz)	6.03, 1.85	1.88 (3H, dq, $J=7.4, 1.6$ Hz)	1.84, 6.04
2	6.03 (1H, qq, $J=7.2, 1.3$ Hz)	1.85, 1.94	6.04 (1H, qq, $J=7.4, 1.2$ Hz)	1.84, 1.88
4	1.85 (3H, dq, $J=1.3, 1.6$ Hz)	6.03, 1.94	1.84 (3H, dq, $J=1.2, 1.6$ Hz)	6.04, 1.88
6	4.84 (2H, dq, $J=1.1, 1.0$ Hz)	6.48, 2.12	4.76 (2H, dq, $J=1.2, 1.1$ Hz)	6.41, 2.05
8	6.48 (1H, tq, $J=1.1, 7.1$ Hz)	2.12, 4.84	6.41 (1H, tq, $J=1.2, 7.2$ Hz)	2.05, 4.76
9	2.12 (3H, dt, $J=7.1, 1.0$ Hz)	6.48, 4.84	2.05 (3H, dt, $J=7.2, 1.1$ Hz)	6.41, 4.76
11	4.82 (2H dd, $J=6.6, 1.2$ Hz)	6.61, 6.18	4.74 (2H, dd, $J=6.5, 1.4$ Hz)	6.54, 6.15
12	6.18 (1H, dt, $J=15.6, 6.6$ Hz)	6.61, 4.82	6.15 (1H, dt, $J=15.8, 6.5$ Hz)	6.54, 4.74
13	6.61 (1H, dt, $J=15.6, 1.2$ Hz)	6.18, 4.82	6.54 (1H, dt, $J=15.8, 1.4$ Hz)	6.15, 4.74
15	6.94 (1H, d, $J=2.3$ Hz)	6.92	7.24 (2H, d, $J=8.4$ Hz)	6.78
16			6.78 (2H, d, $J=8.4$ Hz)	7.24
18	6.81 (1H, d, $J=8.5$ Hz)	6.92	6.78 (2H, d, $J=8.4$ Hz)	7.24
19	6.92 (1H, dd, $J=8.4, 2.3$ Hz)	6.81, 6.94	7.24 (2H, d, $J=8.4$ Hz)	6.78
20	3.88 (3H, s)			
21	3.90 (3H, s)		3.74 (3H, s)	

(*Z*)-2-[(*Z*)-2-methyl-2-butenoyloxy methyl]butenoate.

Compound **2** had a molecular formula of $\text{C}_{20}\text{H}_{24}\text{O}_5$, as determined by elemental analysis and eims (molecular peak at m/z 344). The ^{13}C -nmr spectrum showed twenty signals which were attributed, on the basis of DEPT experiments, to $\text{Me}\times 3$, $\text{MeO}\times 1$, $\text{OCH}_2\times 2$, $=\text{CH}\times 8$, $=\text{C}\times 4$, and $\text{COO}\times 2$ (Table 1). The structure of this compound was deduced mainly by comparison of the chemical shifts in the ^1H - and ^{13}C -nmr spectra with those of compound **1**. The ^1H -nmr spectrum showed signals located at the same position and having the same multiplicities and J values as those of **1**. The only difference was due to the presence of one MeO group in the aromatic ring. The ABX system of **1** was replaced by an AA'BB' system (two doublets, 2H each, $J=8.4$ Hz, at δ 6.78 and 7.24) in **2**. Complete assignments of the ^1H - and

^{13}C -nmr resonances, and therefore the identification of **2** as (*E*)-3-(4-methoxyphenyl)-2-propen-1-yl (*Z*)-2-[(*Z*)-2-methyl-2-butenoyloxymethyl]butenoate, was accomplished by using HETCOR and COLOC nmr experiments, as described for **1**.

Compounds **3** and **4** appeared to be isomers as evidenced by their elemental analysis data and eims spectra (molecular peak at m/z 208). Their ^1H -nmr spectra showed that both compounds contained a 3,4-dimethoxyphenyl group and that their difference was due to an aliphatic unsaturated side-chain. The side-chain was $-\text{CH}(\text{CH}_3\text{O})-\text{CH}=\text{CH}_2$ in **3**, whereas it was $-\text{CH}=\text{CH}-\text{CH}_2-\text{OCH}_3$ in **4**. Characterization of **3** was confirmed by its ^{13}C -nmr spectral data (see Experimental). All signals were assigned by means of a DEPT pulse experiment and by comparison with data of **1**. Based on the above evidence, **3** was characterized as 1,2-dimethoxy-4-

FIGURE 1. Eims fragmentation pattern for **1**.

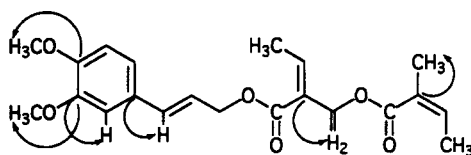


FIGURE 2. COLOC nmr data of compound **1** in CDCl_3 ($J_{\text{CH}} = 7$ Hz).

(1-methoxy-2-propenyl)benzene. Compound **3** was previously isolated from the same plant and showed strong antifeedant activity to *Mythimna unipuncta* Haw. (2).

1,2-Dimethoxy-4-(3-methoxy-1-propenyl)benzene [4], 3,4-dimethoxy cinnamaldehyde [5], and 7-(3,3-dimethylallyloxy)-6-hydroxycoumarin (prenyletin, [6]) were identified by comparing their spectral data with those reported in the literature (3–5).

Analogues of **1** and **2** are not common in nature. Compounds similar to **1** and **2** have been previously isolated only from the family Asteraceae [from *Blumea lacera* (6) and *Aster intricatus* (7)].

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The following instruments were used: nmr, Bruker AC-200 Spectrospin nmr spectrometer; ir, Perkin-Elmer 684 spectrophotometer; optical rotation, measured at 20° using a Perkin-Elmer 141 polarimeter with a 1-dm cell; eims, VG TRIO 2000 (70 eV). Si gel 60 (70–230 mesh and 230–400 mesh, Merck) and Sephadex LH-20 (Pharmacia) were used for cc. Tlc was performed on Si gel 60 precoated layers (Merck).

One and two-dimensional nmr spectra were measured in CDCl_3 , with chemical shifts reported in δ values (ppm) downfield from the TMS internal standard, and coupling constants reported in Hz. The COLOC, DEPT, direct HETCOR, and COSY nmr experiments were carried out using Bruker commercial microprograms. Delays of 2D ^{13}C - ^1H shift correlations by long-range coupling (COLOC) were adjusted to an average CH coupling of 7 Hz to obtain maximum polarization transfer.

PLANT MATERIAL.—The leaves of *Bupleurum fruticosum* were collected in July 1992, near Dorgali, Nuoro, Sardinia, Italy. A voucher specimen is maintained in the Herbarium of the Istituto di Botanica Farmaceutica, Università di Sassari, Italy.

EXTRACTION AND ISOLATION.—Powdered air-dried leaves (500 g) were extracted at room temperature with *n*-hexane. The residue was parti-

tioned with 90% MeOH. The MeOH-soluble part (8.8 g) was concentrated *in vacuo* and a portion (6.0 g) was chromatographed on a Sephadex LH-20 column using MeOH. Five fractions were collected after monitoring by tlc on Si gel using *n*-hexane-EtOAc (7:3). Compounds were detected using $\text{Ce}(\text{SO}_4)_2/\text{H}_2\text{SO}_4$ spray reagent.

Fraction III (3.65 g) was further purified by flash cc on Si gel eluting with *n*-hexane-EtOAc solvent systems to give five fractions: A and B (*n*-hexane-EtOAc, 9:1), C and D (*n*-hexane-EtOAc, 7:3), and E (*n*-hexane-EtOAc, 1:1). Fraction D (770 mg) was chromatographed further by flash cc on Si gel, using toluene- CHCl_3 -EtOAc (80:13:7), to yield compounds **1** (690 mg) and **2** (14 mg).

Fraction B (18 mg) was chromatographed by gravity cc on Si gel, using toluene-EtOAc (93:7), to yield compound **3** (8.8 mg). Compounds **4** (5 mg), **5** (2.5 mg), and **6** (2.5 mg) were obtained from fractions C (46 mg), F (49 mg), and G (25 mg), respectively, using similar isolation schemes.

(*E*)-3-(3,4-DIMETHOXYPHENYL)-2-PROPEN-1-YL (Z)-2-[(Z)-2-METHYL-2-BUTENYLOXYMETHYL] BUTENOATE [**1**].—Pale yellow oil; tlc R_f 0.23 [toluene- CHCl_3 -EtOAc (80:13:7)]; ir ν max 3540, 1718, 1640, 1516, 1265, 1280, 1141, 1028, 945 cm^{-1} ; eims m/z [M^+] 374 (29), 274 (13), 193 (100), 177 (96), 146 (65), 99 (37), 83 (95), 82 (88), 56 (79); ^1H - and ^{13}C -nmr data, see Tables 1 and 2; *anal.*, found C 67.11, H 6.77; $\text{C}_{21}\text{H}_{26}\text{O}_6$ requires C 67.37, H 6.95.

(*E*)-3-(4-METHOXYPHENYL)-2-PROPEN-1-YL (Z)-2-[(Z)-2-METHYL-2-BUTENYLOXYMETHYL] BUTENOATE [**2**].—Light yellow oil; tlc R_f 0.38 [toluene- CHCl_3 -EtOAc (80:13:7)]; ir ν max 3540, 1718, 1640, 1516, 1265, 1280, 1141, 1028, 945 cm^{-1} ; eims m/z [M^+] 344 (0.4), 244 (3), 163 (38), 147 (49), 115 (22), 91 (33), 83 (93), 82 (62), 55 (100), 54 (54), 53 (37), 39 (48); ^1H - and ^{13}C -nmr data, see Tables 1 and 2; *anal.*, found C 69.43, H 6.71, $\text{C}_{20}\text{H}_{24}\text{O}_5$ requires C 69.76, H 6.97.

1,2-DIMETHOXY-4-(1-METHOXY-2-PROPENYL)BENZENE [1'-METHOXYMETHYLEUGENOL] [**3**].—Yellowish oil; tlc R_f 0.15 [toluene- CHCl_3 -EtOAc (80:13:7)]; $[\alpha]_D^{25} + 6.5^\circ$ ($c = 0.2$); ir ν max 1680, 1602, 1585, 1440, 1265, 1082, 980, 900 cm^{-1} ; eims m/z [M^+] 208 (41), 177 (100), 146 (55), 131 (21), 91 (33), 77 (29), 55 (25), 15 (14); ^1H nmr (CDCl_3) δ 3.32, 3.87 and 3.89

(3H each, s, OMe \times 3), 4.57 (1H, dt, $J=6.5$ and 1.0 Hz, H-1), 5.24 (2H, m, H-3), 5.93 (1H, ddd, $J=6.5$, 10.1 , and 17.0 Hz, H-2), 6.85 (1H, d, $J=2.3$ Hz, H-2'), 6.86 (1H, d, $J=8.3$ Hz, H-5'), 6.88 (1H, dd, $J=2.3$ and 8.3 Hz, H-6'); ^{13}C nmr (CDCl_3) δ 56.5 (OMe \times 2), 59.0 (OMe), 85.1 (C-1), 110.2 (C-2'), 111.5 (C-5'), 116.8 (C-3), 119.9 (C-6'), 134.0 (C-1'), 139.4 (C-2), 149.1 and 149.7 (C-3' and C-4'); *anal.*, found C 68.85, H 7.23; $\text{C}_{12}\text{H}_{16}\text{O}_3$ requires C 69.23, H 7.69.

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