



FLAVANS AND A-TYPE PROANTHOCYANIDINS FROM *PRUNUS PROSTRATA*

A. R. BILIA,* I. MORELLI, M. HAMBURGER†‡ and K. HOSTETTMANN†

Dipartimento di Chimica Bioorganica, Università di Pisa, via Bonanno 33, I-56126, Pisa, Italy; †Institut de Pharmacognosie et Phytochimie, Université de Lausanne, BEP, CH-1015, Lausanne-Dorigny, Switzerland

(Received 12 January 1996)

Key Word Index—*Prunus prostrata*; Rosaceae; aerial parts; 5,7,2',5'-tetrahydroxy-(2R,3R)-flavan-3-ol; 7-methoxy-(+)-catechin; ent-epiafzelechin ($2\alpha \rightarrow O \rightarrow 7$, $4\alpha \rightarrow 8$) quercetin.

Abstract—A new natural dimeric A-type proanthocyanidin and two new monomeric flavanols have been isolated from *Prunus prostrata* and identified as ent-epiafzelechin ($2\alpha \rightarrow O \rightarrow 7$, $4\alpha \rightarrow 8$) quercetin, 5,7,2',5'-tetrahydroxy-(2R,3R)-flavan-3-ol and, 7-methoxy-(+)-catechin, respectively. The structurally related metabolites ent-epiafzelechin ($2\alpha \rightarrow O \rightarrow 7$, $4\alpha \rightarrow 8$) afzelechin, ent-epiafzelechin ($2\alpha \rightarrow O \rightarrow 7$, $4\alpha \rightarrow 8$) kaempferol, ent-epiafzelechin ($2\alpha \rightarrow O \rightarrow 7$, $4\alpha \rightarrow 8$) epicatechin, ent-epiafzelechin ($2\alpha \rightarrow O \rightarrow 7$, $4\alpha \rightarrow 8$) epiafzelechin, ent-epiafzelechin ($2\alpha \rightarrow O \rightarrow 7$, $4\alpha \rightarrow 8$) catechin, (+)-catechin and (–)-epicatechin were also isolated. Structure elucidation was achieved by extensive NMR (homo- and hetero-nuclear two-dimensional correlation, NOE difference, and selective INEPT experiments) and CD studies. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Prunus prostrata Labill. (Rosaceae, subfamily Prunoideae) is a Mediterranean species. In Italy, it is found only in Sardinia where it grows, as a small creeping shrub, in garigues and on rocky soils [1]. The plant is known locally as 'Carisedda agreste' and the fruits are used in the folk medicine for gastrointestinal disturbances. Our previous phytochemical investigation of the aerial parts led to the isolation and identification of 16 compounds. Among them, two coumarins, a reduced β -ionone, and a benzoic acid derivative were new natural products [2]. As a result of our continuing studies on the phenolic constituents of *P. prostrata*, we now report the isolation and structure elucidation of eight flavanols and proanthocyanidins, among them one new A-type proanthocyanidin dimer and two flavan-3-ol derivatives.

RESULTS AND DISCUSSION

The aerial parts of *Prunus prostrata* were defatted with *n*-hexane and subsequently extracted in a Soxhlet apparatus with chloroform and chloroform–methanol (9:1). Preliminary fractionation of the extracts was achieved by a gel filtration on Sephadex LH-20. Compounds 1–8 were subsequently isolated from the

phenolic fractions by a combination of gel chromatography on Sephadex LH-20 and reversed-phase low- and high-pressure liquid chromatography (see Experimental).

From the chloroform extract, 5,7,2',5'-tetrahydroxy-(2R,3R)-flavan-3-ol (1) and 7-methoxy-(+)-catechin (2) were isolated by HPLC RP-18. The ^1H and ^{13}C NMR spectral data of both molecules were indicative of flavan-3-ols [3].

Compound 1 had a molecular weight of 290 amu, as established by D/CI mass spectrometry ($[\text{M} + \text{H}]^+$ and $[\text{M} + \text{NH}_4]^+$ at m/z 291 and 308, respectively). Along with signals attributable to an epicatechin moiety (δ 4.81 (1H, *d*, $J < 1$ Hz, H-2), δ 4.17 (1H, *m*, H-3), δ 2.73 (1H, *dd*, $J = 16.0$ and 2.9 Hz, H-4a), and δ 2.88 (1H, *dd*, $J = 16.0$ and 4.2 Hz, H-4b)), the ^1H NMR showed an AB spin system due to ring A (δ 5.91 (H-6) and δ 5.93 (H-8)). An A_2B spin system [*brs* at δ 6.77 (2H, H-3' and H-4'), δ 6.97 (1H, *s*, H-6')] suggested a B ring substituted at C-2 and C-5; this was confirmed by the ^{13}C NMR spectral data [4]. The vicinal coupling constants of the heterocyclic C ring were in agreement with a *cis* relative substitution at C-2 and C-3. By comparison of the optical rotation and CD spectrum with data reported for (–)-epicatechin [5, 6], the structure of compound 1 was established as 5,7,2',5'-tetrahydroxy-(2R,3R)-flavan-3-ol.

Compound 2 had a molecular weight of 304 amu, as established by D/CI MS ($[\text{M} + \text{H}]^+$ and $[\text{M} + \text{NH}_4]^+$ at m/z 305 and 322). In addition to signals attributable to the C ring of a catechin moiety (δ 4.56 (1H, *d*, $J = 7.6$ Hz, H-2), at δ 3.98 (1H, *m*, H-3), and two *dd*

*Author to whom correspondence should be addressed.

‡Present address: Centre for Natural Product Research, Institute of Molecular and Cell Biology, National University of Singapore, Singapore 0511.

(1H each) at δ 2.50 (J = 16.1 and 8.2 Hz) and δ 2.84 (J = 16.1 and 5.4 Hz)), the ^1H NMR spectrum exhibited resonances of five aromatic protons including an AB spin system (δ 5.85 and δ 5.97) with a meta coupling (J = 1.9 Hz, H-6 and H-8) and an ABX system attributable to a 3',4' disubstituted B ring. A singlet at δ 3.96 (3H) was indicative of one aromatic methoxyl group. The linkage of this substituent at C-7 was established by a NOESY experiment (NOE between the signal at 3.96 and the signals of H-6 and H-8) and from the ^{13}C NMR spectral data. Compared with the ^{13}C NMR spectrum of catechin, the C-7 resonance of compound **2** was shifted downfield by 4.4 ppm, while the signals attributable to the adjacent C-6 and C-8 were shielded of 1.3 ppm. The *trans* substitution at C-2 and C-3 was established from the magnitude of the vicinal coupling constants ($J_{2,3}$ = 7.8 Hz), and the absolute configuration (2*R*, 3*R*) was deduced from the optical rotation and CD measurements in comparison with the data reported for (+)-catechin [5, 6]. Thus, **2** is 7-methoxy-(+)-catechin.

From the chloroform-methanol extract, the proanthocyanidins ent-epiafzelechin ($2\alpha \rightarrow O \rightarrow 7$, $4\alpha \rightarrow 8$) quercetin (**3**), ent-epiafzelechin ($2\alpha \rightarrow O \rightarrow 7$, $4\alpha \rightarrow 8$) kaempferol (**4**), ent-epiafzelechin ($2\alpha \rightarrow O \rightarrow 7$, $4\alpha \rightarrow 8$) afzelechin (**5**), ent-epiafzelechin ($2\alpha \rightarrow O \rightarrow 7$, $4\alpha \rightarrow 8$) ent-epicatechin (**6**), ent-epiafzelechin ($2\alpha \rightarrow O \rightarrow 7$, $4\alpha \rightarrow 8$) ent-epiafzelechin (**7**), and ent-epiafzelechin ($2\alpha \rightarrow O \rightarrow 7$, $4\alpha \rightarrow 8$) catechin (**8**), were also isolated. Compounds **3**–**8** exhibited ^1H and ^{13}C NMR spectral data which were indicative for A-type proanthocyanidins. Diagnostic features in all ^1H NMR spectra were the presence of an AB system in the heterocyclic proton region (δ 5.50–4.00; $J_{3,4}$ = 3.2–3.7 Hz) and an isolated aromatic proton (H-6 of ring D) [7, 8].

The ^{13}C NMR spectrum of compound **3**, $\text{C}_{30}\text{H}_{20}\text{O}_{12}$ (FAB-mass spectrum: $[\text{M} + \text{H}]^+$ at m/z 573), showed the presence of three aliphatic carbons ($\text{CH} \times 1$, $\text{CH-O} \times 1$, $\text{O-C-O} \times 1$), 26 olefinic carbons ($\text{CH} \times 10$, $\text{C} \times 5$, $\text{C-O} \times 11$), and one carbonyl carbon (Table 1). In the IR spectrum, the absorption bands at 1655 cm^{-1} and

3380 cm^{-1} were characteristic of a conjugated and chelated carbonyl and of hydroxyl groups, respectively.

The ^1H NMR and DQ PSCOSY spectra revealed the presence of two methine (AB, δ 4.20 and 4.99, J = 3.2 Hz, H-3, H-4 of ring C) and 10 aromatic protons. The presence of a tetra- and a penta-substituted aromatic ring was deduced from the presence of meta-coupled doublets (δ 5.94 and δ 6.05; J = 2.3 Hz, H-6, H-8 of ring A) and an isolated proton singlet (δ 6.30, H-6 of ring D). The remaining seven protons appeared as A_2B_2 and ABX patterns attributable to *p*-substituted and 1,3,4-trisubstituted aromatic rings (rings B and E). A positive Mg/HCl test and the UV spectrum recorded in MeOH (λ_{max} at 232, 279, 332, and 379 nm) suggested the presence of a flavanol moiety [9]. A bathochromic shift of the absorption maximum at 379 nm (band I) in the presence of AlCl_3 (+53 nm) was in agreement with a free hydroxyl at C-3 of the flavanol moiety, while the absorption at 279 nm (band II) indicated a free 5-hydroxyl [10]. The presence of a 'lower' quercetin moiety was confirmed by comparison of the ^{13}C NMR chemical shifts of the ring B and C carbons of **3** with those of authentic quercetin, whereas the remaining NMR signals were in agreement with an 'upper' 5,7,4'-trihydroxy-flavan-3-ol. The ($4 \rightarrow 8$, $2 \rightarrow O \rightarrow 7$)-interflavanyl linkage characteristic of A-type proanthocyanidins was corroborated by NMR spectral evidences: proton and carbon chemical shifts for the D-ring methine (δ 6.30 and δ 97.9) were in agreement with the chemical shifts of CH-6' (δ 6.2–6.4 and δ 97–100) but not with that of CH-8' (δ 6.5–6.9 and δ 94–96) of quercetin and other flavonols [10, 11]. In addition, presaturation of the H-3 resonance of the 'upper' flavanyl moiety led to NOE of the signals attributable to H-6 of ring D and H-2' and H-6' of ring B (Fig. 1). This confirmed the spatial proximity of these nuclei, which is in accord with a ($2\alpha \rightarrow O \rightarrow 7$, $4\alpha \rightarrow 8$)-linkage and an equatorial H-3 [8]. Additional NOEs observed are shown in Fig. 1.

The absolute configurations at C-2 and C-4 of **3** were established by CD measurements. The bounds between

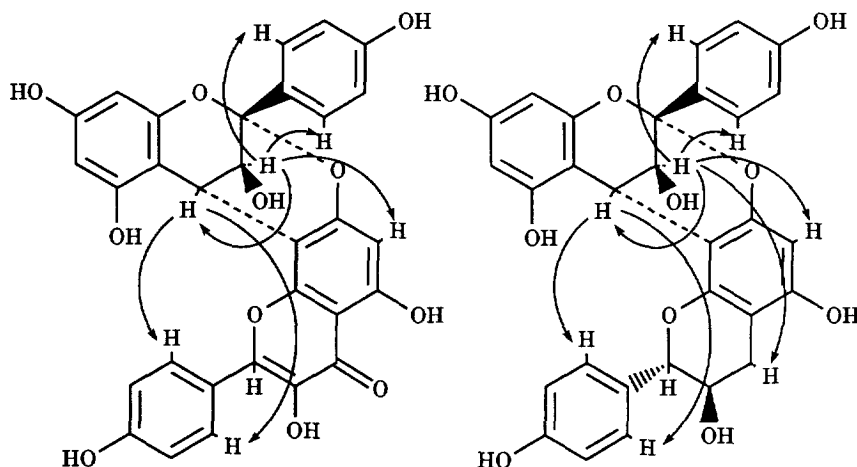


Fig. 1. NOEs observed for compounds **3** and **5**.

Table 1. ^1H and ^{13}C NMR data of compounds **3** and **5** (200 MHz, CD_3OD , TMS as internal standard)

H	3	5	C	3	5
			2	100.6 (C)	100.5 (C)
3	4.20 (<i>d</i> , $J = 3.2$ Hz)	4.12 (<i>d</i> , $J = 3.7$ Hz)	3	67.7 (CH)	67.6 (CH)
4	4.99 (<i>d</i> , $J = 3.2$ Hz)	4.23 (<i>d</i> , $J = 3.7$ Hz)	4	29.3 (CH)	29.3 (CH)
			5	156.1 (C)	156.1 (C)
6	5.94 (<i>d</i> , $J = 2.3$ Hz)	5.92 (<i>d</i> , $J = 2.3$ Hz)	6	99.1 (CH)	98.1 (CH)
			7	158.2 (C)	158.2 (C)
8	6.05 (<i>d</i> , $J = 2.3$ Hz)	6.05 (<i>d</i> , $J = 2.3$ Hz)	8	95.8 (CH)	96.5 (CH)
			9	156.7 (C)	156.7 (C)
			10	105.3 (C)	104.0 (C)
			11	130.2 (C)	130.2 (C)
12	7.52 (<i>d</i> , $J = 8.7$ Hz)	7.50 (<i>d</i> , $J = 7.6$ Hz)	12	130.3 (CH)	130.0 (CH)
13	6.85 (<i>d</i> , $J = 8.7$ Hz)	6.82 (<i>d</i> , $J = 7.6$ Hz)	13	116.3 (CH)	116.3 (CH)
			14	156.7 (CH)	158.9 (C)
15	6.85 (<i>d</i> , $J = 8.7$ Hz)	6.82 (<i>d</i> , $J = 7.6$ Hz)	15	116.3 (CH)	116.3 (CH)
16	7.52 (<i>d</i> , $J = 8.7$ Hz)	7.50 (<i>d</i> , $J = 7.6$ Hz)	16	130.3 (CH)	130.0 (CH)
2'		4.76 (<i>d</i> , $J = 8.2$ Hz)	2'	146.9 (C)	83.9 (C)
3'		4.08 (<i>ddd</i> , $J = 8.2, 8.9$ and 5.5 Hz)	3'	135.6 (C)	68.4 (CH)
H ₂ -4'		2.56 (<i>dd</i> , $J = 16.3$ and 8.9 Hz)	4'	176.1 (C)	29.3 (CH ₂)
		3.01 (<i>dd</i> , $J = 16.3$ and 5.5 Hz)			
			5'	160.9 (C)	156.1 (C)
6'	6.30 (<i>s</i>)	6.08 (<i>s</i>)	6'	97.9 (CH)	96.5 (CH)
			7'	159.5 (C)	154.1 (C)
			8'	102.7 (C)	106.6 (C)
			9'	154.2 (C)	152.2 (C)
			10'	107.6 (C)	102.9 (C)
			11'	122.3 (C)	131.6 (CH)
12'	8.13 (<i>m</i>)	7.36 (<i>d</i> , $J = 7.5$ Hz)	12'	115.5 (CH)	129.5 (CH)
13'		6.82 (<i>d</i> , $J = 7.5$ Hz)	13'	145.7 (C)	115.5 (CH)
			14'	147.5 (C)	158.9 (C)
15'	6.92 (<i>d</i> , $J = 8.5$ Hz)	6.82 (<i>d</i> , $J = 7.5$ Hz)	15'	115.8 (CH)	115.5 (CH)
16'	8.13 (<i>m</i>)	7.36 (<i>d</i> , $J = 7.5$ Hz)	16'	120.5 (CH)	129.5 (CH)

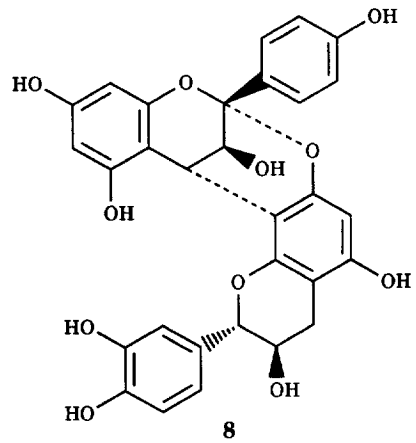
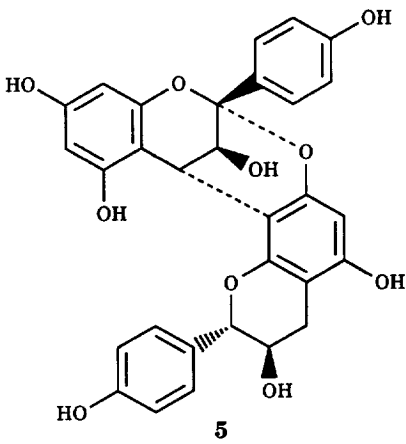
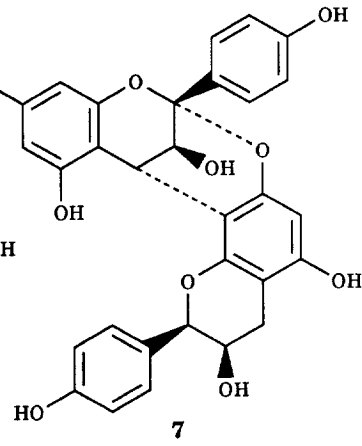
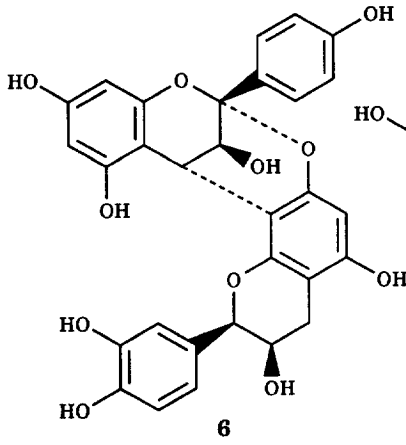
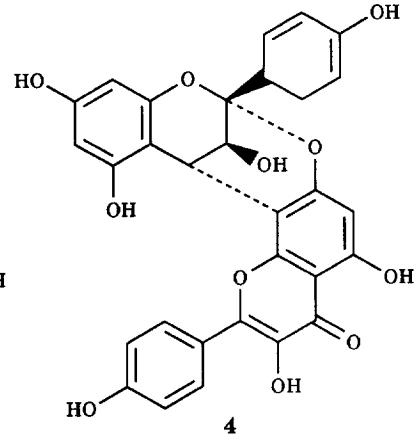
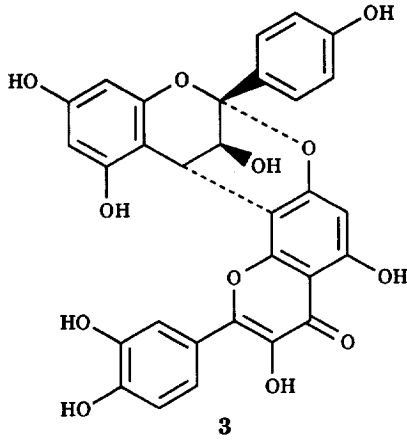
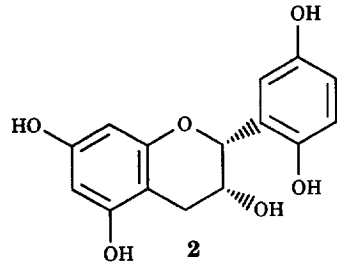
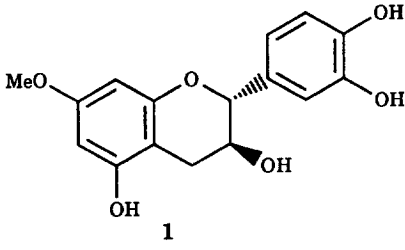
the two units of A-type proanthocyanidins must be *cis* [6, 12], and the strong negative Cotton effect in the region between 200 and 230 nm ($[\theta]_{232} - 138468$, $[\theta]_{204} + 110918$) indicated the ($2\alpha,4\alpha$)-configuration [8]. Because the hydroxyl group at C-3 (ring C) was in axial (β)-position, the absolute configuration was $2S,3S,4S$ and therefore compound **3** is ent-epiafzelechin ($2\alpha \rightarrow O \rightarrow 7$, $4\alpha \rightarrow 8$) quercetin.

The structure of compound **4** (ent-epiafzelechin ($2\alpha \rightarrow O \rightarrow 7$, $4\alpha \rightarrow 8$) kaempferol) was established by comparison of spectral data (UV, IR, CD, mass and NMR) with those reported in the literature [13]. This compound was previously isolated from the roots of *Ephedra* plants. Compound **3** is the second example of an A-type proanthocyanidin containing a flavanol moiety.

Compound **5**, $\text{C}_{30}\text{H}_{24}\text{O}_{10}$ (D/C1 mass spectra: $[\text{M} + \text{H}]^+$ and $[\text{M} + \text{NH}_4]^+$ at m/z 545 and 562), exhibited a ^{13}C NMR spectrum with resonances for six aliphatic carbons ($\text{CH}_2 \times 1$, $\text{CH} \times 1$, $\text{CH-O} \times 3$, $\text{O-C-O} \times 1$) and 24 olefinic carbons ($\text{CH} \times 11$, $\text{C} \times 5$, $\text{C-O} \times 8$) (Table 1). The ^1H and ^{13}C NMR, including homo- and heteronuclear correlation experiments (DQ PSCOSY, HETCOR) were in accordance with a structure of A-type composed of two 5,7,4'-trihydroxy flavanol units. Because H-2 and H-3 were *trans*-oriented ($J_{2,3} = 8.2$ Hz) the 'lower unit' was identified as afzelechin. The stereochemistry at C-3 of the 'upper' flavanyl moiety

was established by NOE difference measurements as for proanthocyanidin **3** (Fig. 1). Determination of the relative configuration of C-2', C-3', and C-4' was achieved by selective INEPT and NOE experiments. A high-amplitude negative Cotton effect in the diagnostic wavelength region of the CD spectrum [14, 15] indicated also for the proanthocyanidin **5** the ($2\alpha,4\alpha$)-configuration. Above all, the weak positive couplet at 270 nm showed an α substitution at C-2 of ring F [12]. Thus, compound **5** is ent-epiafzelechin ($2\alpha \rightarrow O \rightarrow 7$, $4\alpha \rightarrow 8$) afzelechin, in accordance with the revised form of compound **Pla** [16], a metabolite isolated from the roots of peach (*P. persica* Batsh.) [17]. The structures of the other A-type proanthocyanidins **6** (ent-epiafzelechin ($2\alpha \rightarrow O \rightarrow 7$, $4\alpha \rightarrow 8$) epicatechin) [18], **7** (ent-epiafzelechin ($2\alpha \rightarrow O \rightarrow 7$, $4\alpha \rightarrow 8$) epiafzelechin) [8], and **8** (ent-epiafzelechin ($2\alpha \rightarrow O \rightarrow 7$, $4\alpha \rightarrow 8$) catechin) [8], were established by comparison of spectral data (UV, IR, CD, MS and NMR) with those reported in the literature.

A series of such compounds, including **5**–**8**, have been characterized from other *Prunus* species such as *P. spinosa* L. [8], *P. persica* [16] and *P. jacquemontii* Hook [18]. Moreover, compound **5** is a plant growth inhibitor flavanol isolated from the roots of peach (*P. persica*) [16], suggesting the flavanols to be one of the chemical factors causing soil sickness often encountered in peach and other Rosaceae cultivations.



EXPERIMENTAL

General. ^1H and ^{13}C NMR spectra were recorded in CD_3OD operating at 200 MHz for ^1H and 50 MHz for ^{13}C . Chemical shifts are given in δ (ppm) with TMS as int. standard. DEPT, double-quantum filtered phase-sensitive COSY (DQ PSCOSY), and HETCOR spectra were recorded using standard microprogrammes. The soft ^1H pulse for selective INEPT [19] experiments was optimized according to ref. [20]. The delay settings were optimized for $^1J_{\text{CH}} = 4$ or 8 Hz. TLCs were carried out on silica gel 60 F_{254} gel precoated Al sheets and RP-18 HPTLC plates (Merck). Compounds were visualized at UV 254 nm, and by spraying the Godin reagent. HPLC analysis of the frs was carried with a Hewlett-Packard 1090 series II instrument equipped with a photodiode array detector. The conditions were as follows: Li-ChroCART RP-18 column (7 μm , 3.9×150 mm i.d.; Merck); mobile phases $\text{CH}_3\text{CN}-\text{H}_2\text{O}$ 1:4 with 0.1% TFA (System 1) or 3:17 with 0.1% TFA (System 2) in isocratic conditions; flow rate 1.0 ml min^{-1} ; detection at 270 nm. Semiprep. HPLC was carried out on a Eurospher 80 RP-18 (7 μm , 16×250 mm i.d.; Knauer) using the same eluents as for analytical HPLC. Flow rate was 10 ml min^{-1} detection at 270 nm. Low-pressure chromatography was carried out on a Lobar RP-18 column (31×2.5 cm; Merck) with $\text{MeOH}-\text{H}_2\text{O}$ (3:2) as eluent. The flow rate was $1-1.5 \text{ ml min}^{-1}$.

Plant material. The aerial parts of *P. prostrata* (leaves, stems, bark, and flowers) were collected at Dorgali in the province of Nuoro (Sardinia, Italy) in May 1991. The plant material was identified by Dott. Salvatore Mele (Azienda Foreste Demaniali Regione Sardegna, via Trieste, Nuoro, Italy). A dried voucher specimen is deposited in the Dipartimento di Chimica Bioorganica, University of Pisa (Italy).

Extraction and isolation of phenolic compounds. Dried ground aerial parts (700 g) were defatted with *n*-hexane and extracted in a Soxhlet apparatus sequentially with CHCl_3 and $\text{CHCl}_3-\text{MeOH}$ (9:1). Portions of the CHCl_3 (3 g) and $\text{CHCl}_3-\text{MeOH}$ (4.9 g) extracts were sepd on Sephadex LH-20 columns (50×4 cm and 75×6 cm i.d., respectively) with MeOH as eluent. Frs of 10 ml and 20 ml, respectively, were collected and analysed by TLC on silica gel ($\text{CHCl}_3-\text{MeOH}$, 17:3 and 4:1). Fr. 14 (320 mg) from the CHCl_3 extract was further purified over a Cromabond RP-18 precolumn (1 g) eluted with 5 ml of $\text{MeOH}-\text{H}_2\text{O}$ (2:3). The residue (100 mg) was fractionated by semiprep. HPLC ($\text{MeCN}-\text{H}_2\text{O}$ 3:17) to yield **1** (15 mg) and **2** (54 mg). From fr. 15, (–)-epicatechin (56 mg) was obtained by crystallization from MeOH . Semiprep. HPLC ($\text{MeCN}-\text{H}_2\text{O}$, 3:17) of fr. 16 yielded (+)-catechin. Fr. 14 (250 mg) of the $\text{CHCl}_3-\text{MeOH}$ extract was further purified by RP-LPLC ($\text{MeOH}-\text{H}_2\text{O}$ 3:2) to yield compounds **3** (20 mg) and **4** (18 mg). Fr. 21 (250 mg) was sepd on a Sephadex LH-20 column (3×50 cm i.d.) into six fractions (I–VI) with MeOH as mobile phase. Semiprep. HPLC of fraction III (50 mg) ($\text{MeCN}-\text{H}_2\text{O}$ 1:4) yielded compounds **6** (25 mg) and **7** (18 mg),

while HPLC separation of fraction V (62 mg) under the same conditions gave **7** (24 mg) and **5** (30 mg). Fr. 23 (100 mg) of the $\text{CHCl}_3-\text{MeOH}$ extract was purified by HPLC ($\text{MeCN}-\text{H}_2\text{O}$ 1:4) to yield compound **8** (40 mg).

Compound 1. $\text{C}_{15}\text{H}_{14}\text{O}_6$; $k' = 2.39$ (System 1); UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 272; $[\alpha]_{\text{D}} -22$ ($c = 0.3$, Me_2CO); D/CI MS (positive ion mode): m/z 308 $[\text{M} + \text{NH}_4]^+$ and 291 $[\text{M} + \text{H}]^+$; CD: $[\theta]_{240} +9200$, $[\theta]_{272} -9500$; ^1H NMR (CD_3OD): δ 2.73 (1H, *dd*, $J = 16.0$ and 2.9 Hz, H-4a), 2.88 (1H, *dd*, $J = 16.0$ and 4.2 Hz, H-4b), 4.17 (1H, *m*, H-3), 4.81 (1H, *d*, $J < 1$ Hz, H-2), 5.91 (1H, *d*, $J = 1.9$ Hz, H-6), 5.93 (1H, *d*, $J = 1.9$ Hz, H-8), 6.77 (2H, *s*, H-3' and H-4'), 6.97 (1H, *s*, H-6'); ^{13}C NMR (CD_3OD): ppm 29.2 (C-4), 67.3 (C-3), 79.0 (C-2), 96.5 (C-8), 96.9 (C-6), 100.6 (C-10), 115.3 (C-3'), 115.6 (C-4'), 119.3 (C-6'), 132.5 (C-1'), 145.7 (C-2' and C-5'), 157.9 (C-5), 157.6 (C-9), 158.2 (C-7).

Compound 2. $\text{C}_{16}\text{H}_{16}\text{O}_6$; $k' = 1.45$ (System 1); UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 270; $[\alpha]_{\text{D}} +15$ ($c = 0.6$, Me_2CO); D/CI MS (positive ion mode): m/z 322 $[\text{M} + \text{NH}_4]^+$ and 305 $[\text{M} + \text{H}]^+$; CD: $[\theta]_{226} -15700$, $[\theta]_{242} +2200$, $[\theta]_{280} -5300$; ^1H NMR (CD_3OD): δ 2.50 (1H, *dd*, $J = 16.1$ and 8.2 Hz, H-4b), 2.84 (1H, *dd*, $J = 16.1$ and 5.4 Hz, H-4a), 3.96 (3H, *s*, OMe), 3.98 (1H, *m*, H-3), 4.56 (1H, *d*, $J = 7.6$ Hz, H-2), 5.85 (1H, *d*, $J = 1.9$ Hz, H-6), 5.97 (1H, *d*, $J = 1.9$ Hz, H-8), 6.74 (2H, *m*, H-5' and H-6'), 6.84 (1H, *d*, $J = 1.9$ Hz, H-2'); ^{13}C NMR (CD_3OD): ppm 28.4 (C-4), 55.0 (OMe), 68.8 (C-3), 82.7 (C-2), 94.0 (C-8), 94.7 (C-6), 102.7 (C-10), 115.1 (C-2'), 115.8 (C-5'), 120.0 (C-6'), 132.0 (C-1'), 145.1 (C-3' and C-4'), 157.0 (C-5), 156.9 (C-9), 160.8 (C-7).

Compound 3. $\text{C}_{30}\text{H}_{20}\text{O}_{12}$; $k' = 4.05$ (System 2); UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 232, 279, 332, 379, + AlCl_3 : 234, 277, 432; CD: $[\theta]_{232} -138468$, $[\theta]_{204} +110918$; FAB MS (positive ion mode): m/z 573 $[\text{M} + \text{H}]^+$; IR $\nu_{\text{max}}^{\text{NaCl}}$ cm^{-1} : 3380, 1625, 1655, 1520, 1250, 1150; ^1H and ^{13}C NMR: see Table I.

Compound 5. $\text{C}_{30}\text{H}_{24}\text{O}_{10}$; $k' = 6.02$ (System 2); UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 222, 273; CD: $[\theta]_{220} -132165$, $[\theta]_{203} +137907$; D/CI MS (positive ion mode): m/z 562 $[\text{M} + \text{NH}_4]^+$ and 545 $[\text{M} + \text{H}]^+$; IR ν_{max} (NaCl) cm^{-1} : 3400, 1630, 1530, 1240, 1150; ^1H NMR and ^{13}C NMR: see Table I.

Acknowledgements—Financial support for this research project was provided by MURST (Ministero dell'Università e della Ricerca Scientifica e Tecnologica, Roma). We are grateful to Dott. C. Bertucci and Prof. P. Salvadori (Dipartimento di Chimica e Chimica Industriale) for the CD measurements, and to Dr J-L. Wolfender (Institut de Pharmacognosie et Phytochimie) for the D/CI mass spectra.

REFERENCES

1. Pignatti, S. (1982) *Flora d'Italia*, vol. I, p. 167. Edagricole, Bologna.
2. Bilia, A. R., Cecchini, C., Marsili, A., Mele, S. and

- Morelli, I. (1993) *J. Nat. Prod.* **56**, 2142.
3. Senda, Y., Ishiyama, Y., Imaizynii, S. and Hanaya, K. (1977). *J. Chem. Soc. Perkin Trans.* **1**, 217.
4. Agrawal, P. K. (1989) *Carbon-13 NMR of Flavonoids*, Elsevier Science Publishers B. V., Amsterdam.
5. Porter, L. J., Newman, R. H., Foo, L. Y., Wong, H. and Hemingway, R. W. (1982) *J. Chem. Soc. Perkin Trans. 1*, 1217.
6. Weinges, K., Baehr, W., Ebert, W., Goeritz, K. and Marx, H. D. (1969) *Fortschr. Chem. Org. Naturst.* **27**, 158.
7. Jacques, D., Haslam, E., Bedford, G. R. and Greatbanks, D. (1974) *J. Chem. Soc. Perkin Trans. 1*, 2263.
8. Kolodziej, H., Sakar, M. K., Burger, J. F. W., Engelshowe, R. and Ferreira, D. (1991) *Phytochemistry* **30**, 2041.
9. Markham, K. R., Ternay, B., Stanley, R., Geiger, H. and Mabry, T. J. (1978) *Tetrahedron* **34**, 1389.
10. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer Verlag, Berlin.
11. Chiari, V. M., Ahmad, S. and Osterdahl, B.-G. (1978) *Z. Naturforsch.* **3**, 1547.
12. Hikino, H., Shimoyama, N., Kasahara, Y., Takahashi, M. and Konno, C. (1982) *Heterocycles* **19**, 1381.
13. Hikino, H., Takahashi, M. and Konno, C. (1982) *Tetrahedron Letters* **23**, 673.
14. Botha, J. J., Ferreira, D. and Roux, D. G. (1978) *J. Chem. Soc., Chem. Comm.*, 689.
15. Botha, J. J., Young, D. A., Ferreira, D. and Roux, D. G. (1981) *J. Chem. Soc. Perkin Trans. 1*, 1213.
16. Porter, L. J. (1994) in *The Flavonoids: Advances in Research since 1986* (Harborne, J. B., ed.), pp. 45–47. Chapman & Hall, Cambridge.
17. Ohigashi, H., Minami, S., Fukui, H., Koshimizu, K., Mizutani, F., Sugiura, A. and Tomana, T. (1982) *Agric. Biol. Chem.* **46**, 2555.
18. Pant, G., Nautiyal, A. K., Rawat, M. S. M., Sutherland, J. K. and Morris, G. A. (1992) *Magn. Res. Chem.* **30**, S142–S147 (Special Issue).
19. Bax, A. (1984) *J. Magn. Res.* **57**, 314.
20. Radics, L. and Sandor, P. (1988) *Magn. Moments* **3**(4), 2.