Constituents of Prunus spinosa

Ana Crespo Irizar, Margarita Fernandez Fernandez, Antonio G. González, and Angel Gutierrez Ravelo

J. Nat. Prod., **1992**, 55 (4), 450-454• DOI: 10.1021/np50082a008 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 55, No. 4, pp. 450-454, April 1992

CONSTITUENTS OF *PRUNUS SPINOSA*

ANA CRESPO IRIZAR, MARGARITA FERNANDEZ FERNANDEZ,

Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad de Navarra, Pamplona, Spain

ANTONIO G. GONZÁLEZ,* and ANGEL GUTIERREZ RAVELO

CPNO Antonio González, Instituto de Bio-Orgánica, Universidad de La Laguna, Carretera La Esperanza 2, La Laguna, 38206 Tenerife, Canary Islands, Spain

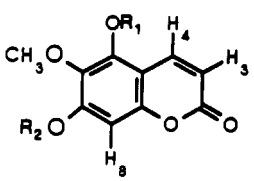
ABSTRACT.—Five known flavanoids, kaempferol 3,7-dirhamnoside, kaempferol, quercetin, catechin, and epicatechin have been obtained from *Prunus spinosa* together with the new compound, 5-hydroxy-6-methoxy-7-*O*-β-D-glucosyl coumarin [**1**], and a recently isolated type A proanthocyanidin dimer, *ent*-epicatechin-(2α→7,4α→8)-catechin [**2**]. The absolute configuration of the latter was determined by means of cd studies and application of the Horeau method.

Over a vast expanse of the northeastern Iberian peninsula, sloes from the Blackthorn *Prunus spinosa* L. (Rosaceae) (1) are macerated to make a schnapps called "pacharan" (2) and are decocted for use in folk medicine as an antihypertensive agent.

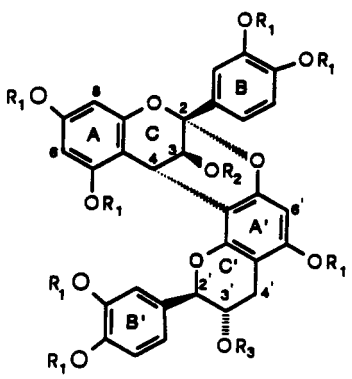
This plant species is widespread throughout Europe and north Africa, being found from 0 to 1500 m above sea level. Its phytochemistry has already been studied (3), yielding flavonol heterosides (quercetin and kaempferol) (4) and the coumarins aesculetin (5), umbelliferone, and scopoletin. These flavonols have been shown to possess hypocholesterolemic, spasmolytic, antitoxic, anti-inflammatory, vitamin P, positive inotropic, diuretic, and natriuretic properties (3,6).

The proanthocyanidins are a class of secondary phenolic metabolites consisting of units of flavan-3-ol bound by one or two interflavan bonds. Those with twice-bonded structures, type A, are not often found in nature, and their distribution is limited to species of Ericaceae, Hippocastanaceae, Lauraceae, and Rosaceae. Proanthocyanidin A-2 is the best known and most abundant (7).

We report here the isolation and structural elucidation of a previously undescribed



1 R_1=H, R_2=glc
3 R_1=H, R_2=$(Ac)_4$glc
4 R_1=Ac, R_2=$(Ac)_4$glc



2 R_1=R_2=R_3=H
5 R_1=Me, R_2=R_3=Ac
6 R_1=Me, R_2=Ac, R_3=H
7 R_1=Me, R_2=H, R_3=Ac
8 R_1=Me, R_2=R_3=H
9 R_1=Me, R_2=Ac, R_3=2-C_6H_5-C_3H_7CO
10 R_1=Me, R_2=2-C_6H_5-C_3H_7CO, R_3=Ac

coumarin and a known type A proanthocyanidin dimer based on a spectroscopic study of their derivatives and the determination of the absolute configuration of the proanthocyanidin by application of the Horeau method and a cd study.

RESULTS AND DISCUSSION

The *n*-BuOH fraction of the aqueous extract obtained by decoction of dry young branches of *P. spinosa* was subjected to repeated chromatography on Sephadex LH-20 and Si gel to afford a series of compounds including kaempferol 3,7-dirhamnoside, kaempferol, quercetin, catechin, and epicatechin, together with **1** and **2**, the structures of which were determined on the basis of the following data.

Compound **1** was isolated as a crystalline solid with the molecular ion at *m/z* $[M]^+$ 370 and a fragment clearly visible at *m/z* 208 (88% relative intensity) corresponding to the coumarin moiety of the molecule. In the ^{1}H-nmr spectrum, signals for the coumarin protons H-3 and H-4 were observed as doublets at δ 6.36 and 8.01 ($J = 9.8$ Hz), respectively, and for an aromatic proton as a broad singlet at δ 6.94. Double resonance experiments indicated that H-4 was coupled with the aromatic proton at δ 6.94. In view of the low J value this was most likely a long-distance coupling between H-4 and H-8; these protons possess the distance and spatial disposition required for this type of coupling. Signals for a three-proton singlet for an aromatic MeO at δ 3.95 and a collection of signals typical of the glucose moiety were also observed. A meticulous nOe (Figure 1) identified this compound as a 5-hydroxy-6-methoxy-7-*O*-β-D-glucoside. The nature of the glucoside moiety was determined by gc comparison after hydrolysis and the preparation of silyl derivatives.

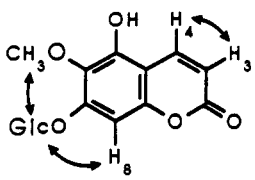


FIGURE 1. Compound **1**: nOe experiment.

When compound **1** was acetylated with Ac_2O in pyridine at room temperature for 24 h, products **3** and **4** were obtained. Their data agree with the structure proposed (see Experimental). To the best of our knowledge this is the first time that 5-hydroxy-6-methoxy-7-*O*-β-D-glucosyl coumarin [**1**] has been described.

Compound **2** was isolated as its heptamethyl diacetate **5** when the fractions containing it and testing negative for the presence of -MeO were treated with CH_2N_2 at −18° for 3 days and then acetylated with Ac_2O in pyridine for 24 h.

Compound **5**, mp 115, had a molecular formula of $C_{41}H_{42}O_{14}$ (hrms). Its ^{1}H-nmr spectrum displayed signals for two acetate methyls at δ 1.75 and 1.91 and for seven aromatic MeO, thus establishing the existence of two hydroxy groups on an aliphatic moiety and seven phenolic groups, which indicated that there were three oxygenated groups in the molecule corresponding to ether bridges. The ^{13}C-nmr spectrum exhibited signals for six aliphatic carbons corresponding to a CH_2 at δ 25.53, four carbons at δ 25.92, 67.79, 69.89, and 78.51, a tetra-substituted carbon at δ 97.86, and signals for 24 aromatic carbons, nine of which were methine carbons.

The study of the shifts of the aromatic protons showed signals forming part of an AB system as two doublets centered at δ 6.00 and 6.27 with $J = 2.3$ Hz, and for an aromatic proton as a singlet at δ 6.20. These values are similar to those given in the literature for phloroglucinol (8) and its derivatives, so it can be deduced that **5** has two oxygenated tetra- and penta-substituted benzene rings in the relative positions 1, 3, and 5.

The six remaining aromatic protons were observed between δ 6.89 and 7.29, forming part of complex ABC type systems requiring two tri-substituted rings in the relative positions 1, 3, and 4 in the molecule of **5**. These data and comparison of the spectrum with those of epicatechin and some of its derivatives revealed similar coupling patterns.

In the aliphatic proton region of the ^{1}H-nmr spectrum of **5**, signals centered at δ 2.60 and 3.05 were observed as two double doublets (J = 16.0, 8.0 Hz) of one proton each coupled in turn with a doublet signal at δ 5.40 (J = 8.0 Hz) which was also coupled with another doublet centered at δ 4.90 (J = 8.0 Hz) forming an AMX system. Two doublets of an AB system at δ 4.88 and 5.51 (J = 3.5 Hz) were also seen.

From these data it may be concluded that there must be two units of flavanol in the molecule joined together by two bonds. The determination of the type of bonding and stereochemistry was the next step as three natural proanthocyanidins A1 (7), A6 (9), and A7 (9) are known to have unions with one bridge in ether form, while dioxan-linked profisetidin (10) has a union with two ether bridges. The possibility of a dioxan-bridge-type union in this instance was discounted, as such a union would not have any $-CH_2-$ groups. The choice of linkage type (2↦7, 4↦8), (2↦7, 4↦6), or (2↦5, 4↦6) was made on the basis of a study of the chemical shift of the MeO on C-5, which is at δ 3.29 in **5** and thus very similar to that of A1 (7) where it appeared at δ 3.32 with a (2↦7, 4↦8) linkage. The other linkage types invariably exhibit shifts for all the MeO's between δ 3.70 and 4.00. Dreiding models clearly showed that the B′ ring is very close to the MeO on C-5 which accounts for this sort of shift. Once the type of linkage had been determined, the lower flavanol unit was identified as being that of the AMX system, a unit of catechin, while that of the epicatechin AB system was found on the upper part. The cd curve of **5** showed a coupling in the short-wave area (11) with values $[\theta]_{220} -2.3 \times 10^5$, $[\theta]_{206} +3.8 \times 10^5$ clearly indicating that the configuration of C-4 was 4*S*. Thus the absolute configuration of the upper part was determined as *ent*-epicatechin. To determine the absolute configuration of the lower unit, the monoacetates **6** (free hydroxyl at C′-3) and **7** (free hydroxyl at C-3) and diol **8** were prepared by hydrolysis. The Horeau method (12) could then be applied, as a secondary alcohol was involved, and this method has proved useful for these compounds (13) while the Prelog method (14) has not. The Horeau method applied to **6** and **7** gave two derivatives **9** and **10**. The respective residual acids both showed negative [α]D; thus, the absolute configuration of the upper unit was confirmed and that of the lower unit determined, characterizing compound **2** as *ent*-epicatechin (2↦7, 4↦8) catechin following the nomenclature of Barret *et al.* (11).

While this paper was in preparation Kolodziej *et al.* (15) reported isolating from the flowers of the same vegetable species a product with data coinciding with those of **5**. Assessment of the stereochemistry of the C′ ring by the Horeau method confirmed the form that was previously assigned (15) on the basis of nOe data. The stereochemistry of the lower unit, however, although in fact correct, had not been firmly established, as no tests were made with a compound of the opposite stereochemistry. Information is included in this paper about five derivatives not prepared in that study (15) (Table 1).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The ^{1}H and ^{13}C nmr were collected on a Bruker WP-200SY spectrometer at 200 and 50 MHz, respectively, with $CDCl_3$, C_6D_6, or CD_3OD as solvents and TMS as internal standard. The ir spectra were taken on a Perkin-Elmer 681 spectrophotometer using $CHCl_3$, film, or KBr. The uv spectra were recorded on a Perkin-Elmer 550SE spectrophotometer with MeOH as solvent. Ms were run on a VG Micromass ZAB-2F, and cd on a JASCO 600 spectropolarimeter with MeCN as solvent. The optical activity was measured on a Perkin-Elmer 241 polarimeter with $CHCl_3$. The mp's were determined using an Olympia BHS microscope with a Mettler FP 82 hot plate coupled to a Mettler FP 80 processor. Preparative tlc was developed on pre-coated Schleicher and Schüll F-1500/LS 254 foils.

TABLE 1. ^{1}H-nmr Data of Compounds **5–10**.[a]

Proton	Compound					
	5	**6**	**7**	**8**	**9**	**10**
H-3	5.51 d (3.5)	5.52 d (3.5)	4.31 d (3.5)	4.30 d (3.5)	5.52 d (3.5)	5.49 d (3.5)
H-4	4.88 d (3.5)	4.83 d (3.5)	4.95 d (3.5)	4.88 d (3.5)	4.80 d (3.5)	4.90 d (3.5)
H-6	6.27 d (2.3)	6.27 d (2.3)	6.27 d (2.3)	6.27 d (2.3)	6.27 d (2.3)	6.30 d (2.3)
H-8	6.00 d (2.3)	5.99 d (2.3)	6.00 d (2.3)	5.99 d (2.3)	5.98 d (2.3)	6.00 d (2.3)
H-2′	4.90 d (8.0)	4.57 d (8.0)	4.83 d (8.0)	4.55 d (8.0)	4.77 d (8.0)	4.90 d (8.0)
H-3′	5.40 c (8.0)	4.22 c (8.0)	5.40 c (8.0)	4.27 c (8.0)	5.35 c (8.0)	5.20 c (8.0)
H-4′	2.60 dd 3.05 dd (16,8)	2.60 dd 3.08 dd (16,8)	2.60 dd 3.16 dd (16,8)	2.56 dd 3.14 dd (16,8)	2.60 dd 3.10 dd (16,8)	2.60 dd 3.05 dd (16,8)
H-6′	6.20 s	6.20 s	6.20 s	6.21 s	6.19 s	6.19 s

[a]δ (ppm). Figures in parentheses are *J* (Hz).

PLANT MATERIAL.—*P. spinosa* (8.7 kg) was collected in Navarra in March 1986, and voucher specimens are on file in the Herbarium of the Department of Botany, Facultad de Farmacia, Universidad de Navarra, Pamplona, Spain. Dried young branches were prepared with boiling H_2O, and the resulting extract was treated with *n*-BuOH to give a *n*-BuOH extract (250 g) which was chromatographed on Sephadex LH-20 (40 g) and then on Si gel to give kaempferol, quercetin, kaempferol 3,7-dirhamnoside, catechin, epicatechin, and compounds **1** and **2**.

5-HYDROXY-6-METHOXY-7-*O*-β-D-GLUCOSYL COUMARIN [**1**].—Compound **1** was isolated as white sheet crystals (8 mg): mp 135°; uv λ max (MeOH) nm 206, 328; ir ν max cm^{-1} 3360, 2900, 1710, 1610, 1550, 1450, 1370, 1250, 1040, 990, 820; eims *m/z* (% rel. int.) $[M]^+$ 370 (1), 209 (11), 208 (88), 194 (11), 193 (100), 165 (25), 137 (28), 95 (12), 81 (8), 73 (8), 69 (30), 67 (10), 66 (5), 63 (6), 60 (12), 57 (9), 55 (7), 53 (11), 51 (5); ^{1}H nmr (CD_3OD) δ 3.30–3.73 (6H, overlapping signals), 3.88 (3H, s), 5.05 (1H, d, J = 7.4 Hz), 6.16 (1H, d, J = 9.7 Hz), 6.62 (1H, s), 8.24 (1H, d, J = 9.7 Hz). To identify the sugar, acid hydrolysis was performed. The acid mixture was extracted with EtOAc, with the coumarin in the organic phase and the sugar in the aqueous phase. This sugar was then identified by gc, and its retention time (Rt) agreed with that of reference glucose.

ACETYLATION OF 5-HYDROXY-6-METHOXY-7-*O*-β-D-GLUCOSYL COUMARIN [**1**].—A fraction containing compound **1** was treated with Ac_2O in pyridine for 24 h at room temperature. After preparative tlc, the tetraacetate **3** (10 mg) and the pentaacetate **4** (20 mg) of **1** were separated.

Compound **3** was obtained as a white amorphous powder: ^{1}H nmr ($CDCl_3$) δ 1.97 (3H, s), 2.02 (3H, s), 2.04 (3H, s), 2.08 (3H, s), 3.67 (1H, m), 3.86 (3H, s), 3.99 (1H, dd, J = 2.5, 12.4 Hz), 4.23 (1H, dd, J = 5.0, 12.4 Hz), 5.16–5.31 (4H, superimposable), 6.22 (1H, d, J = 9.7 Hz), 6.78 (1H, s), 7.93 (1H, d, J = 9.7 Hz).

Compound **4** was also obtained as a white amorphous powder: eims *m/z* (% rel. int.) $[M - 249]^+$ 331 (13), 279 (10), 271 (4), 208 (5), 193 (4), 169 (49), 167 (20), 157 (5), 151 (5), 150 (11), 149 (88), 147 (6), 145 (7), 141 (5), 131 (5), 127 (17), 126 (5), 121 (7), 117 (6), 115 (9), 113 (12), 112 (8), 110 (7), 109 (41), 107 (6), 105 (9), 103 (7), 101 (5), 99 (11), 98 (11), 97 (22), 96 (8), 95 (12), 93 (7), 91 (7), 86 (9), 85 (32), 84 (16), 83 (26), 71 (53), 69 (36), 60 (12), 57 (100), 55 (57); ^{1}H nmr ($CDCl_3$) δ 1.99 (3H, s), 2.03 (3H, s), 2.04 (3H, s), 2.08 (3H, s), 2.38 (3H, s), 3.66 (1H, m), 3.79 (3H, s), 3.99 (1H, dd, J = 2.4, 12.4 Hz), 4.24 (1H, dd J = 5.0, 12.4 Hz), 5.11–5.35 (4H, superimposable) 6.36 (1H, d, J = 9.8 Hz), 6.94 (1H, s), 8.01 (1H, d, J = 9.8 Hz); (C_6D_6) δ 1.62 (3H, s), 1.63 (3H, s), 1.73 (3H, s), 1.74 (3H, s), 1.77 (3H, s), 2.76 (1H, m), 3.53 (3H, s), 3.63 (1H, dd, J = 2.0, 12.5 Hz), 4.05 (1H, dd, J = 4.6, 12.5 Hz), 4.96 (1H, d, J = 8.1 Hz), 5.22–5.34 (2H, superimposable), 5.50 (1H, t, J = 8.6 Hz), 6.05 (1H, d, J = 9.8 Hz), 6.61 (1H, s), 7.63 (1H, d, J = 9.8 Hz).

ent-EPICATECHIN-(2α→7,4α→8)-CATECHIN HEPTAMETHYL ETHER DIACETATE [**5**].—Obtained as a white amorphous powder by methylation and subsequent acetylation of a fraction of the general chromatography: hrms $[M]^+$ 758.2471 (calcd for $C_{41}H_{42}O_{14}$, 758.2460); ir ν max cm^{-1} 2900, 1730, 1600, 1500, 1450, 1220, 1130, 1020, 800, 750; eims *m/z* (% rel. int.) $[M+1]^+$ 759 (1), 698 (1), 639 (1), 547 (1), 536 (1), 494 (1), 385 (1), 343 (30), 222 (3), 212 (6), 181 (24), 167 (19), 165 (12), 162 (4), 151 (13), 57 (100); ^{1}H nmr ($CDCl_3$) δ 1.75 (3H, s), 1.91 (3H, s), 3.29 (3H, s), 3.72 (3H, s), 3.75 (3H, s), 3.89 (3H, s), 3.90 (3H × 2, s), 3.91 (3H, s), 6.86–7.29 (6H, superimposable), see Table 1; ^{13}C nmr ($CDCl_3$) δ 20.89 (q), 21.07 (q), 25.53 (t), 25.92 (d), 55.03 (q), 55.45 (q), 55.61 (q), 56.00 (2C, q), 56.18 (2C, q), 67.79 (d), 69.89 (d), 78.51 (d), 92.14 (d), 92.65 (d), 93.31 (d), 97.86 (s), 102.31 (s), 104.52 (s), 106.10 (s), 109.96 (d), 110.32 (d), 110.62 (d), 110.96 (d), 119.63 (d), 119.38 (d), 130.64 (s), 130.81 (s), 148.56 (s), 148.99 (s), 149.74 (s), 150.97 (s), 151.61 (s), 153.32 (s), 157.28 (s), 158.40 (s), 159.78 (s), 169.47 (s), 169.86 (s); cd $[\theta]_{206}$ $+3.8\times10^5$, $[\theta]_{220}$ -2.3×10^5, $[\theta]_{249}$ $+0.3\times10^5$, $[\theta]_{271}$ $+0.2\times10^5$, $[\theta]_{287}$ -0.6×10^4.

SELECTIVE HYDROLYSIS OF **5**.—K_2CO_3 (120 mg) was dissolved in C_6H_6-MeOH (5:1) and added to **5** (22 mg), and the reaction was left overnight at room temperature with continual stirring. Compounds **6** (20%), **7** (20.5%), and **8** (24.6%) and unreacted starting material **5** (28.6%) were obtained.

3-Acetyl-heptamethyl ether **6**.—^{1}H nmr ($CDCl_3$) δ see Table 1, 1.75 (3H, s), 3.19 (3H, s), 3.75 (3H, s), 3.86 (3H × 2, s), 3.91 (3H, s), 3.92 (3H, s), 3.94 (3H, s), 6.85–7.37 (6H, superimposed signals).

3'-Acetyl-heptamethyl ether **7**.—^{1}H nmr ($CDCl_3$) δ see Table 1, 1.90 (3H, s), 3.24 (3H, s), 3.74 (3H, s), 3.75 (3H, s), 3.91 (3H, s), 3.92 (3H × 2, s), 3.94 (3H, s), 6.87–7.36 (6H, superimposed signals).

Heptamethyl ether **8**.—^{1}H nmr ($CDCl_3$) δ see Table 1, 3.13 (3H, s), 3.74 (3H, s), 3.76 (3H, s), 3.92 (3H, s), 3.93 (3H × 2, s), 3.95 (3H, s), 6.93–7.35 (6H, superimposed signals).

Application of the Horeau method to **6** and **7** gave the derivatives **9** and **10**, respectively; the ^{1}H nmr data are given in Table 1. In both cases, [α]D of the residual acid taken in C_6H_6 proved negative: −0.14 and −0.5.

Compound **9**.—^{1}H nmr ($CDCl_3$) δ see Table 1, 1.73 (3H, s), 3.16 (1H, s), 3.69–3.94 (superimposed signals), 6.77–7.36 (superimposed signals).

Compound **10**.—^{1}H nmr ($CDCl_3$) δ see Table 1, 1.90 (3H, s), 3.12 (3H, s), 3.69–3.93 (superimposed signals), 6.74–7.94 (superimposed signals).

ACKNOWLEDGMENTS

We thank Prof. A. San Feliciano for the nOe experiments and Prof. J. Trujillo for the cd spectra. We are indebted to the AIETI, CICYT (Grant No. FAR88-501) and EEC [011*0505ES(JR)] for subsidies.

LITERATURE CITED

1. T.G. Tutin, V.H. Heywood, N.A. Burges, D.M. Moore, D.H. Valentine, S.M. Walters, and D.A. Webb, Eds., "Flora Europaea," Cambridge University Press, 1968, Vol. 2, p. 78.
2. M. Fernández, "Las Plantas en La Medicina Popular, 1. Navarra Húmeda del Noroeste," Estudios Vascos Eusko Ikaskuntza, Pamplona, 1981, p. 33.
3. V.A. Makarov, *Rastit. Resur.*, **8**, 42 (1972).
4. V. Ivanov and N. Nikolov, *Farmatsiya*, **19**, 36 (1969).
5. R.D.H. Murray, Jesus Méndez, and Stewart A. Brown, "The Natural Coumarins: Occurrence, Chemistry, and Biochemistry," J. Wiley and Sons, New York, 1982, p. 517.
6. V.A. Makarov, *Farmacol. Toxicol.*, **32**, 438 (1969).
7. G. Nonaka, S. Morimoto, J. Kinjo, T. Nohara, and I. Nishioka, *Chem. Pharm. Bull.*, **35**, 149 (1987).
8. P.G. Waterman and D.F. Faulkner, *Planta Med.*, **37**, 178 (1979).
9. S. Morimoto, G. Nonaka, and I. Nishioka, *Chem. Pharm. Bull.*, **35**, 4717 (1987).
10. D.A. Young, D. Ferreira, and D.G. Roux, *J. Chem. Soc., Perkin Trans. 1*, 2031 (1983).
11. M.W. Barret, W. Klyne, P.M. Scopes, A.C. Fletcher, L.J. Porter, and E. Haslam, *J. Chem. Soc., Perkin Trans. 1*, 2375 (1979).
12. A. Horeau and H.B. Kagan, *Tetrahedron*, **20**, 2431 (1964).
13. H. Hikino, N. Shimoyama, Y. Kasahara, M. Takahashi, and C. Konno, *Heterocycles*, **19**, 1381 (1982).
14. A.J. Birch, J.W. Clark-Lewis, and A.V. Robertson, *J. Chem. Soc.*, 3586 (1957).
15. H. Kolodziej, M.K. Sakar, J.F.W. Burger, R. Engelshowe, and D. Ferreira, *Phytochemistry*, **30**, 2041 (1991).

Received 14 August 1991