Phytochemistry, Vol. 31, No. 4, pp. 1432-1434, 1992
Printed in Great Britain.

0031-9422/92 $5.00+0.00

TYPE A PROANTHOCYANIDINS FROM *PRUNUS SPINOSA*

ANTONIO G. GONZÁLEZ,* A. CRESPO IRIZAR,† A. G. RAVELO and MARGARITA FERNÁNDEZ FERNÁNDEZ†

C.P.N.O. Antonio González, Instituto de Bio-Orgánica Universidad de La Laguna, Carretera La Esperanza 2, La Laguna, 38206 Tenerife, Canary Islands, Spain; †Departamento de Farmacia y Tecnología Farmaceutica Facultad de Farmacia, Universidad de Navarra, Pamplona, Spain

(*Received* 14 *August* 1991)

Key Word Index—*Prunus spinosa*; Rosaceae; proanthocyanidins; mahuannin A; *ent*-epiafzelechin-(2α→7, 4α→8)-epicatechin.

Abstract—Two proanthocyanidins were isolated from the branches of *Prunus spinosa* and identified as the known compounds, mahuannin A and *ent*-epiafzelechin-(2α→7,4α→8)-epicatechin. The structures were identified from spectroscopic data and CD studies. Hitherto undescribed derivatives are described and the ^{13}C NMR data were analysed using C–H bidimensional correlations and selective INEPT techniques.

INTRODUCTION

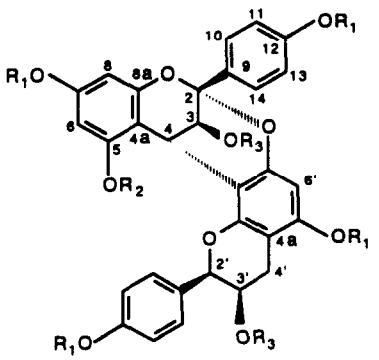
Prunus spinosa [1] (sub-family Prunoideae, section Euprunus) is distributed throughout Europe and the Middle East. It is widespread in Spain, but its ethnobotanic use is best known in Navarra, where infusions of its branches are used in the treatment of hypertension [2] and its macerated fruit in the preparation of a type of schnapps.

The isolation from an extract of young branches of *P. spinosa* and the structural elucidation of two type A proanthocyanidins is described in the present paper. Type A proanthocyanidins have a double interflavanol bond and should more properly be described as propelargonidins according to the nomenclature proposed by Porter [3].

RESULTS AND DISCUSSION

Compound **1** proved to be identical to mahuannin A, obtained by Hikino [4] from the roots of *Ephedra*. Only the data for **1** and partial data for a derivative were reported and were based on CD studies [5] and the application of Horeau's method [6]. Our paper gives information on three derivatives not previously described, mahuannin A tetramethyl ether derivative **2**, the structure of which was determined by a careful spectral study, **3**, the triacetate of **2** and mahuannin A peracetate (**4**), characterised by its ^{1}H (Table 1) and ^{13}C NMR signals unequivocally assigned by DEPT experiments and bidimensional C–H and other correlations (Table 2).

While this paper was being written up, Ferreira *et al.* [7] reported on the constituents of an extract of the flowers of *P. spinosa* describing the isolation of mahuannin A and the proanthocyanidin (propelargonidin) *ent*-epiafzelechin-(2α→7,4α→8)-epicatechin (**5**), identical to one of the products isolated here from an extract of the branches. They gave the structure and absolute configuration based in the main on NOE experiments and CD studies. Weak NOE effects were used [7] to determine the placement of the lower and upper units and the absolute configuration of the lower unit once that of the upper unit had been determined by CD studies.

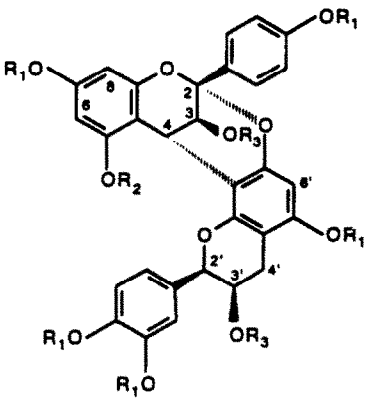


	R_1	R_2	R_3
1	H	H	H
2	CH_3	H	H
3	CH_3	Ac	Ac
4	Ac	Ac	Ac

We, however, have determined the position of the upper unit from the data obtained for the peracetate **7** by means of a selective INEPT experiment [8] involving the irradiation of the H-10 and H-14 protons on the epiafzelechin unit; carbons sited on three bonds were observed and the assignment of the C-2 at δ97.82 was unequivocal with a single shift due to the presence of two oxygens, one phenyl, one carbon bearing an ester on a secondary alcohol, and the aromatic carbons C-10 and C-14 at δ128.27 and C-12 at 151.56. The other ^{13}C NMR data are given in Table 2. The pentamethyl ether **6** was also prepared; its relevant ^{1}H NMR data are also given in Table 1.

Even though data variations are to be expected, given the nature of the different methodologies applied, using compound **5** as reference, the ratio of product obtained

*Author to whom correspondence should be addressed.



	R_1	R_2	R_3
5	H	H	H
6	CH_3	H	H
7	Ac	Ac	Ac

per amount of plant material are of the same order in both cases. Further study is required to allow conclusions to be drawn about the use and distribution of metabolites in the different parts of the plant.

EXPERIMENTAL

^1H and ^{13}C NMR spectra were measured at 200 and 50 MHz, respectively, in $CDCl_3$ or C_6D_6 as solvents with TMS as int. standard. IR spectra were recorded in $CHCl_3$ or as films, UV spectra in MeOH and CD in MeCN. Optical activity was measured in $CHCl_3$ soln. Mps: uncorr. Prep. TLC was done on precoated Schleicher and Schüll F-1500/LS 254 foils.

Extraction and fractionation. Prunus spinosa L. (8.7 kg of branches) was collected in Navarra in March 1986 and voucher specimens are on file in the Herbarium of the Department of Botany, Universidad de Navarra, Pamplona, Spain. Branches were extracted with boiling H_2O and the resulting extract was treated with *n*-BuOH to give an extract (250 g) which was repeatedly chromatographed on Sephadex LH-20 (40 g) and then on silica gel, to give **1** and **5**.

ent-*Epiafzelechin*-(2α→7.4α→8)-*epiafzelechin* 7,12,5′,12′-*tetramethyl ether* (**2**). Obtained as an amorphous solid (12 mg) when a fr. of the general chromatography was treated with CH_2N_2 at −18° for 72 hr. $[\alpha]_D^{20}$ −87.5° ($CHCl_3$; *c* 0.16 g ml^{-1}). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 2900, 1600, 1500, 1450, 1240, 1170, 1120, 1020, 970. ^1H NMR ($CDCl_3$) δ: 6.91 (1H, *s*, OH), 7.0 (2H, *d*, *J* = 8.8 Hz), 7.03 (2H, *d*, *J* = 8.7 Hz), 7.53 (2H, *d*, *J* = 8.7 Hz), 7.68 (2H, *d*, *J* = 8.8 Hz); for rest of signals see Table 1.

ent-*Epiafzelechin*-(2α→7,4α→8)-*epiafzelechin* 7,12,5′,12′-*tetramethyl ether*-3,5,3′-*triacetate* (**3**). Obtained when **2** was treated with Ac_2O in pyridine at room temp. overnight. $[\alpha]_D^{20}$ −28.69° ($CHCl_3$; *c* 0.23 g ml^{-1}). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2900, 1720, 1600, 1500, 1360, 1240, 1130, 1030, 820, 750. ^1H NMR ($CDCl_3$) δ: 1.36 (3H, *s*), 1.78 (3H, *s*), 1.96 (3H, *s*), 3.72 (3H, *s*), 3.73 (3H, *s*), 3.82 (3H, *s*), 3.84 (3H, *s*), 6.92 (2H, *d*, *J* = 8.6 Hz), 6.93 (2H, *d*, *J* = 8.8 Hz), 7.42 (2H, *d*, *J* = 8.6 Hz), 7.62 (2H, *d*, *J* = 8.8 Hz); for rest of signals see Table 1.

ent-*Epiafzelechin*-(2α→7, 4α→8)-*epiafzelechin-hepta-acetate* (**4**). Obtained as an amorphous solid (17 mg) when a fr. of the general chromatography was acetylated as described for **3**, mp 153.5°. $[\alpha]_D^{20}$ −28.65° ($CHCl_3$; *c* 0.37 g ml^{-1}). UV λ_{max}^{MeOH} nm: 206, 269; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2900, 1750, 1600, 1500, 1430, 1350, 1200, 1050, 1010, 900, 840, 740. HRMS $[M]^+$ (calc. for $C_{44}H_{38}O_{17}$, 838.2142; found, 838.2139). ^1H NMR ($CDCl_3$) δ: 1.33 (3H, *s*), 1.78 (3H, *s*), 1.97 (3H, *s*), 2.25 (3H, *s*), 2.27 (3H, *s*), 2.31 (3H, *s*), 2.32 (3H, *s*), 7.14 (2H, *d*, *J* = 8.5 Hz), 7.15 (2H, *d*, *J* = 8.7 Hz), 7.53 (2H, *d*, *J* = 8.5 Hz), 7.68 (2H, *d*, *J* = 8.7 Hz); for rest of signals see Table 1. ^1H NMR (C_6D_6) δ: 1.42 (3H, *s*), 1.45 (3H, *s*), 1.47 (3H, *s*), 1.57 (3H, *s*), 1.67 (3H, *s*), 1.72 (3H, *s*), 1.73 (3H, *s*), 6.93 (2H, *d*, *J* = 8.5 Hz), 7.10 (2H, *d*, *J* = 8.7 Hz), 7.37 (2H, *d*, *J* = 8.5 Hz), 7.63 (2H, *d*, *J* = 8.7 Hz); for rest of signals see Table 1. ^{13}C NMR: see Table 2. EIMS *m/z* (rel. int.): 839 $[M+1]^+$ (0.2), 754 (1), 736 (1), 712 (1), 694 (3), 652 (5), 610 (5), 577 (1), 568 (3), 535 (3), 398 (6), 397 (11), 354 (23), 313 (37), 312 (46), 271 (39), 270 (72), 257 (7), 242 (8), 163 (5), 149 (12), 139 (10), 121 (46), 111 (5), 107 (40), 84 (12), 71 (17), 60 (6), 43 (100).

To prepare the CD spectrum the compound was purified by HPLC RI detector, semi-prep. column μ-Porasil (10 μ, 30 cm × 0.8 cm)], with hexane–EtOAc (1:2) as mobile phase;

Table 1. ^1H NMR spectral data of compounds **2**–**4** and **6**

H	2	3	4 ($CDCl_3$)	4 (C_6D_6)	6
3	4.34 *m*	4.62 *d*	4.76 *d*	5.37 *d*	4.35 *d*
		(3.80)	(3.90)	(3.83)	(3.74)
4	4.52 *d*	5.48 *d*	5.46 *d*	5.46 *d*	4.51 *d*
	(3.64)	(3.80)	(3.90)	(3.83)	(3.74)
6	6.29 *d*	6.54 *d*	6.76 *d*	6.95 *d*	6.29 *d*
	(2.44)	(2.44)	(2.20)	(2.24)	(2.44)
8	6.10 *d*	6.11 *d*	6.38 *d*	6.65 *d*	6.11 *d*
	(2.44)	(2.44)	(2.20)	(2.24)	(2.44)
2′	5.14 *br s*	5.13 *s*	5.23 *s*	4.67 *s*	4.29 *br s*
3′	4.27 *br*	5.30 *br s*	5.26 *br s*	5.20 *br s*	5.11 *s*
4′	2.86 *dd*	2.82 *dd*	2.85 *m*	2.07 *dd*	2.86 *dd*
	(4.14, 17.62)	(4.26, 16.0)		(4.0, 17.20)	(4.16, 16.76)
	3.01 *dd*	2.96 *dd*		2.44 *dd*	3.00 *dd*
	(2.12, 17.62)	(2.80, 16.0)		(2.50, 17.20)	(2.00, 16.76)
6′	6.25 *s*	6.20 *s*	6.44 *s*	6.58 *s*	6.26 *s*

J (Hz) in parentheses.

Table 2. ^{13}C NMR spectral data of compounds **4** and **7**

C	4*	7†	C	4*	7†
2	97.77 *s*	97.82 *s*	2′	77.82 *d*	77.57 *d*
3	66.96 *d*	66.92 *d*	3′	66.67 *d*	66.55 *d*
4	27.09 *d*	27.16 *d*	4′	25.61 *t*	25.60 *t*
4a	113.66 *s*[a]	113.65 *s*	4a′	109.28 *s*[a]	109.36 *s*
5	149.85 *s*[b]	150.62 *s*[b]	5′	149.85 *s*[b]	149.08 *s*[b]
6	107.32 *d*	107.31 *d*	6′	103.68 *d*	103.87 *d*
7	149.06 *s*[b]	149.87 *s*[b]	7′	150.61 *s*[b]	142.54 *s*[b]
8	109.79 *d*	109.87 *d*	8′	105.35 *s*[a]	105.37 *s*[a]
8a	149.32 *s*[b]	149.33 *s*[b]	8a′	151.04 *s*[b]	142.47 *s*[b]
9	135.44 *s*[c]	136.54 *s*[c]	9′	134.45 *s*[c]	134.45 *s*[c]
10	128.46 *d*	128.27 *d*	10′	128.23 *d*	123.06 *d*[d]
11	121.95 *d*	121.23 *d*	11′	121.21 *d*	153.67 *s*[b]
12	151.53 *s*[b]	151.56 *s*	12′	153.64 *s*[b]	151.35 *s*[b]
13	121.95 *d*	121.23 *d*	13′	121.21 *d*	123.69 *d*[d]
14	128.46 *d*	128.27 *d*	14′	128.23 *d*	125.29 *d*
COMe	19.71	19.83			
	20.60	20.63 (2C)			
	20.74	20.69			
	20.95	20.78			
	21.09	20.91			
	21.16 (2C)	21.13			
		21.20			
C=O–Me	168.62	168.05 (2C)			
	168.77	168.63			
	169.17	168.81			
	169.78	169.16			
	169.86 (2C)	169.88			
	170.14	169.96			
		170.36			

*Data taken from DEPT experiments, bidimensional C–H and other correlations.

†Data from DEPT experiments and/or INEPT studies and correlations.

[a–d]Interchangeable values.

R_t 13.3 min. CD $[\theta]_{204}$ +175 021, $[\theta]_{226}$ −133 216, $[\theta]_{268}$ −50 040, $[\theta]_{283}$ +14 874.

ent-*Epiafzelechin*-(2α→7,4α→8)-*epicatechin* 7,12,5′,11′,12′-*pentamethyl ether* (**6**). Obtained (16 mg) when a fr. of the general chromatography was treated with CH_2N_2 at −18° for 72 hr. $[\alpha]_D^{20}$ −65° ($CHCl_3$; *c* 0.18 g ml^{-1}). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 2900, 1600, 1500, 1450. 1250, 1100, 1010, 750. ^1H NMR ($CDCl_3$) δ: 3.70 (3H, *s*), 3.76 (3H, *s*), 3.91 (3H, *s*), 3.92 (3H, *s*), 3.93 (3H, *s*), 6.90 (1H, *s*, OH), 7.00 (2H, *d*, *J* = 8.8 Hz), 7.00–7.33 (3H, superimposed signals), 7.67 (2H, *d*, *J* = 8.8 Hz).

ent-*Epiafzelechin*-(2α→7,4α→8)-*epicatechin-octa-acetate* (**7**). Obtained when a fr. of the general chromatography was acetylated, mp 142°. $[\alpha]_D^{20}$ −36.09° ($CHCl_3$; *c* 0.41 g ml^{-1}). UV λ_{max}^{MeOH} nm: 208, 268. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2900, 1750, 1600, 1500, 1420, 1350, 1200, 1050, 1010, 900, 750. ^{13}C NMR ($CDCl_3$): see Table 2. FABMS *m/z* (rel. int.): 875 (5), 842 (10), 798 (15), 755 (4), 738 (6), 690 (12), 621 (30), 579 (28), 536 (22), 519 (3).

Acknowledgements—We are indebted to the AIETI, CICYT (Grant No. FAR88-501) and EEC [011*0505ES (JR)] for subsidies.

REFERENCES

1. Tutin, T. G., Heywood, V. H., Burges, N. A., Moore, D. M., Valentine, D. H., Walters, S. M. and Webb, D. A. (1968) *Flora Europea*, Vol. 2, p. 78. Cambridge University Press, Cambridge.
2. Fernández, M. (1981) *Las Plantas en La Medicina Popular. 1. Navarra Húmeda del Noroeste*, Sdad. Estudios Vascos Eusko Ikaskuntza, Pamplona.
3. Porter, L. J. (1988) in *The Flavonoids: Advances in Research since* 1980 (Harborne, J. B., ed.), pp. 21–62. Chapman & Hall, London.
4. Hikino, H., Shimoyama, N., Kasahara, Y., Takahashi, M. and Konno, C. (1982) *Heterocycles* **19**, 1381.
5. Barret, M. W., Klyne, W., Scopes, P. M., Fletcher, A. C., Porter, L. J. and Haslam, E. (1979) *J. Chem. Soc., Perkin Trans I* 2375.
6. Horeau, A. (1962) *Tetrahedron Letters* 965.
7. Kolodziej, H., Sakar, M. K., Burger, J. F. W., Engelshowe, R. and Ferreira, D. (1991) *Phytochemistry* **30**, 2041.
8. Bax, A. (1984) *J. Magn. Res.* **57**, 314.