A Prenyloxycoumarin from *Psiadia dentata*[1)]

Hélène FORTIN,[a] Sophie TOMASI,[a] Philippe JACCARD,[b] Valérie ROBIN,[a] and Joël BOUSTIE*,[a]

UPRES Synthèse et Extraction de Molécules à Visée Thérapeutique Faculté de Pharmacie,[a] 2 avenue du Pr. Léon Bernard, 35043 Rennes Cedex, France and Laboratoire de Bactériologie-Virologie, Faculté de Médecine,[b] 2 avenue du Pr. Léon Bernard, 35043 Rennes Cedex, France. Received September 4, 2000; accepted January 9, 2001

A new coumarin identified as 5-hydroxy-6-methoxy-7-(3-methyl-but-2-enyloxy)-2*H*-1-benzopyran-2-one (isoobtusitin) was isolated from *Psiadia dentata.* This compound showed, *in vitro*, a moderate inhibitory activity against poliovirus and a very weak activity against (HIV), whereas it was inactive against (HSV1), (VSV), and murine tumoral cell lines (3LL, L1210).

Key words *Psiadia dentata*; coumarin; *in vitro* assay; isoobtusutin

In the course of study on *Psiadia dentata* (CASS.) DC, an endemic species of the Reunion Islands used in traditional medicine to treat abscesses,[2)] two methoxylated flavonoids, 3,4′-dimethylkaempferol (ermanin) and 3-methylkaempferol (isokaempferide), have been isolated as a result of a bioassay guided fractionation of the antipoliovirus compounds.[3)] Further investigations of non-flavonoid compounds resulted in the isolation of another active product in minute amounts corresponding to a new 7-prenyloxycoumarin. We report here the isolation and identification of this compound along with the first investigation of its antiviral and cytotoxic activity.

Partition of a $CHCl_3$ extract from dried leaves between hexane and 90% aqueous MeOH, followed by various chromatographic steps, resulted in the isolation of a crystalline solid. This compound (**1**) showed a strong yellow fluorescence under UV light (366 nm), and was thought to be a coumarin as it was positive to KOH/EtOH and ammonia vapour.

The IR spectrum of **1** showed absorption bands at 3296, 3086, 1702 (wide band), 1622, 1566 and 1030 cm^{-1} indicating the presence of a hydroxyl group (probably a phenolic one), a diethylenic CH, a conjugated OCO moiety, ethylenic and aromatic C=C, and a C–O moiety, respectively.

High resolution liquid secondary ion mass spectrometry (HR-LSI-MS) revealed $[M+H]^+$ at *m/z* 277.1076, which corresponds to the molecular formula $C_{15}H_{16}O_5$; the 68 unit difference between $[M+H]^+$ (*m/z* 277) and the base peak (*m/z* 209) corresponded to loss of a C_5H_8 fragment, which could be due to a Mac-Lafferty 6-center translation mechanism. ^{1}H- and ^{13}C-NMR data, which are summarized in Tables 1 and 2, were compared to literature values.[4,5)] The ^{1}H-NMR spectrum indicated signals for two CH_3 groups (δ 1.77, 1.80), one OCH_3 group (δ 3.90), and one hydroxyl group (δ 6.19) exchangeable with D_2O, along with aliphatic and aromatic CH and CH_2 groups (δ 4.62, 5.47, 6.20, 6.44, 7.95). The ^{13}C assignments of **1** were made by ^{1}H-decoupled and -coupled ^{13}C experiments, and the putative position of the various substituents on the aromatic ring was based on the comparison with known coumarins and with calculated values for δ ^{13}C.[6)] Directly C–H bonded carbons could be assigned by analysis of the gated decoupling ^{13}C spectrum (Table 2). The doublets at δ 6.2 and 7.95 (J=9.7 Hz) were attributed to H-3 and H-4 and the deshielded nature of the latter proton suggested the presence of an oxygen function at the C-5 position.[7)] The aromatic CH was shielded by both two *O-ortho* and one *O-para* substituents and could be located at the 6- or 8- position. Selective ^{1}H-irradiations followed by the observation of various modifications of the ^{13}C-coupled NMR signals (Chart 1) led to the measurement of some informative $^xJ_{C-H}$ values (Table 2). For instance, we observed on the gated decoupling ^{13}C spectrum that the irradiation of CH_3-9 at 3.90 simplified the signal at 131.8 ppm by suppression of the ^3J coupling through the *O*-bond. The irradiation at 6.44 caused a similar simplification of both multiplets at 131.8 and 102.4 ppm. The last one corresponding to C-4a, the O–CH_3 substituent could therefore only be located at the C-8 or C-6 position. The J_{C-H} deduced values of other selective decoupling experiments are reported in Table 2.

Table 1. ^{1}H (500 MHz)-NMR Data of Isoobtusitin **1** (Recorded in $CDCl_3$)

Position	δ	Multiplicity	J
3	6.20	d	9.7
4	7.95	d	9.7
OH-5	6.19	s	
8	6.44	s	
9	3.90	s	
10	4.62	d	6.7
11	5.47	t	6.7
13	1.77	s	
14	1.80	s	

Table 2. ^{13}C (75 MHz) and HMBC (500 MHz)NMR Data of Isoobtusitin **1** (Recorded in $CDCl_3$)

Position	δ	Multiplicity	$^xJ_{C-H}$	HMBC
2	161.6	dd	$^2J_{2-3}=5$, $^3J_{2-4}=11$	H-3, H-4
3	111.5	br d	$^1J=173$	
4	138.7	d	$^1J=166$	
4a	102.4	m	$^3J_{4a-8}=5$	H-3, H-4, H-8
5	145.7	s		H-4, H-8
6	131.8	m	$^3J_{6-8}=6$, $^3J_{6-9}=4$	H-8, H-9
7	154.9	m		H-8, H-10
8	93.4	d	$^1J=166$	H-4
8a	151.6	s		H-4, H-8
9	61.3	q	$^1J=145$	
10	66.0	t	$^1J=145$	
11	118.6	br d	$^1J=155$	H-10, H-13, H-14
12	139.1	s		H-10, H-13, H-14
13	18.4	q	$^1J=130$	H-11, H-14
14	25.8	q	$^1J=125$	H-11, H-13

∗ To whom correspondence should be addressed. e-mail: Joel.Boustie@univ-rennes1.fr

© 2001 Pharmaceutical Society of Japan

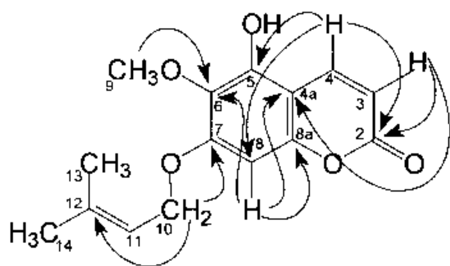

Chart 1. Structure of Isoobtusitin **1** and Relevant HMBC Signals (⌒)

These attributions were confirmed and supplemented by the presence of long-range coupling established with a heteronuclear multiple-bond correlation (HMBC). The respective positions of the three oxygenated substituents on the coumarin ring were unambiguously deduced by the HMBC experiment which showed relevant long-range correlations (Table 2) and by the (NOESY) spectrum. The HMBC spectrum showed interaction between 7.95 ppm and 93.4 ppm, this being assigned to C-8, as the literature reports a W-like coupling between H-4 and C-8.[7] Moreover, the UV spectrum exhibited absorption at 325 nm, typical of 5,7-dioxygenated or 5,6,7-trioxygenated coumarins.[7] These attributions were also confirmed by comparison with chemical shifts of 5-hydroxy-6-methoxy-7-(2,3-dihydroxy-3-methylbutyloxy)-coumarin,[8] a related compound, which only differs from compound **1** by a more oxygenated substituent at the C-7 position, and where the signals at 131.8 and 93.0 were assigned to C-6 and C-8, respectively. The NOESY spectrum showed correlations between H-8 (δ 6.44) and both H-10 (δ 4.62) and H-11 (δ 5.47) of the prenylated chain and no correlation of H-8/CH_3-9 methoxyl singlet. Therefore, compound **1** was identified as 5-hydroxy-6-methoxy-7-(3-methyl-but-2-enyloxy)-2*H*-1-benzopyran-2-one and termed isoobtusitin by reference to the 8-hydroxy isomer previously isolated from *Haplophyllum obtusifolium*.[4]

Coumarins represent an important class of widely distributed *O*-heterocyclic natural products exhibiting many pharmacological properties.[9] To our knowledge, this is the first report of a coumarin in the *Psiadia* genus and the quite unusual 7-*O*-prenylation[9] could be of chemotaxonomic interest. Previous phytochemical studies of these species of Asteraceae reported on flavonoids with various biological activities.[10—12] For *P. dentata*, methoxyflavonoids were proved to be the major antipoliovirus active compounds[3] but isoobtusitin may also contribute to the antipoliovirus effect of the crude extract. Although this compound showed a moderate activity on poliovirus (Table 3), its toxicity on host cells was very low, which led to a better selectivity index (SI=347) than the reference compound, 3-methylquercetin. The antiviral potency of coumarins is now well established and isoobtusitin was also tested for its antiviral effect against herpes virus type 1 (HSV-1) and vesicular stomatitis virus (VSV), but no significant effect was found. It was also evaluated, along with seselin, known to be a naturally occurring pyranocoumarin, for which some synthetic analogs exhibited potent *in vitro* anti-(HIV) activity.[13,14] Only a very weak effect (about 10% inhibition of HIV replication) was observed for these compounds with concentrations close to their cytotoxic concentration 50% (CC_{50}) on Sup T1 cells, *i.e.*, 20 and 80 μg/ml for isoobtusitin and seselin, respectively.

Table 3. *In Vitro* Antipoliovirus Evaluation of Isoobtusitin **1**

Compound	IC_{50} (μM)	CC_{50} (μM)	SI[a]
Isoobtusitin	2.9	1006	347
3-Methylquercetin	0.5	28	56

a) SI: selectivity index$=CC_{50}/IC_{50}$.

Table 4. *In Vitro* Cytotoxicity Evaluation of Isoobtusitin **1** on Murine Tumoral Cells

Compound	IC_{50} (μM)	
	3LL	L1210
Isoobtusitin	106.50	39.60
Doxorubicin	0.10	0.03

The *in vitro* cytotoxic activity of **1** (Table 4) against two murine tumoral cell lines (3LL and L1210) was higher than the criterion ($IC_{50}<4$ μg/ml for a pure compound)[15] to select compounds for further anticancer studies.

Experimental

The melting point was measured on a Kofler Heizbank. IR spectrum was recorded as a KBr pellet on a Perkin–Elmer 16 PC FTIR instrument. ^{1}H-NMR and HMBC (500 MHz) spectra were recorded in $CDCl_3$ using a FT Bruker DMX 500 WB spectrometer, ^{13}C-NMR (75 MHz) and NOESY (300 MHz) spectra were recorded in $CDCl_3$ using a FT Bruker AM 300 WB spectrometer; the chemical shifts (δ) were given in ppm, coupling constants (*J*) were expressed in Hz, using tetramethylsilane (TMS) as internal standard. The LSI-MS mass spectra were obtained with a Varian MAT 311 mass spectrometer. Analytical TLC was performed on precoated Silica gel F_{254} (Alugram®, Art.-Nr. 818 133) plates. After development, the dried plates were examined under short-wave (254 nm) or long-wave (366 nm) UV light and exposed under ammoniacal vapours or sprayed with KOH/EtOH reagent.

The aerial parts of *P. dentata* (Cass.) DC were collected on Reunion Islands in May 1994 and identified by Dr. B. Le Bossé. A voucher specimen is deposited at the Herbarium of the Faculty of Pharmacy-Rennes-France.

Dried powdered leaves (240 g) were continuously extracted with $CHCl_3$ to provide a crude extract (31 g). The dried residue was partitioned between hexane and 90% aqueous MeOH. The aqueous MeOH extract (18 g) was subjected to quick silica gel column chromatography using hexane–EtOAc step gradient ranging from 100 : 0 to 0 : 100 and MeOH. The first collected fractions were rechromatographed on silica gel (35—70 μm; CH_2Cl_2–MeOH 99 : 1, 97 : 3, 50 : 50, MeOH), fractionated over medium pressure liquid chromatography (MPLC) (Si, 35—70 μm; hexane–CH_2Cl_2 60 : 40, hexane–CH_2Cl_2–MeOH 60 : 35 : 5, MeOH) and centrifugal circular chromatography (CCC) (Si, Merck Art-7764, hexane–CH_2Cl_2–MeOH 60 : 38 : 2, CH_2Cl_2–MeOH 95 : 5), and finally purified by HPLC [Kromasil Si column (10 μ), 10×250 mm; mobile phase, CH_2Cl_2–EtOAc 97 : 3; UV detector (264 nm)], followed by a reversed phase HPLC [Kromasil C-18 column (5 μ), 10×250 mm; mobile phase, MeOH/H_2O 70 : 30; UV detector (264 nm)].

Isoobtusitin was obtained as pale yellow needles in MeOH (30 mg); mp 127—130 °C. UV λ_{max} (MeOH) nm<(log ε) 209 (7.7), 325 (7.3). IR ν_{max} (KBr) cm^{-1} 3296, 3086, 1702, 1622, 1566, 1030. ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 75 MHz) see Table 1; HR-LSI-MS *m/z* 277.1076 (Calcd for $C_{15}H_{17}O_5$: 277.1076), *m/z* 209.0449 (Calcd for $C_{10}H_9O_5$: 209.0450), *m/z* 208.0372 (Calcd for $C_{10}H_8O_5$: 208.0378).

The antiviral activity of compound **1** against poliovirus (Sabin II), HSV1 (H29S) and VSV[16] and HIV,[17] and the cytotoxic activity[18] on murine cell lines, L1210 and 3LL were determined by the MTT method. For all the assays, the absorbances were read in an eight-channel computer controlled photometer and data were analysed with the Biolise software. Inhibitory concentration 50% (IC_{50}) and CC_{50} on Vero cells for antipoliovirus assays, CC_{50} on Sup T1 cells for anti-HIV assays, inhibitory concentrations 50%

(IC_{50}) for cytotoxic assays were calculated from the dose–response curves. All the results given in Tables 3 and 4 correspond to the mean of three different experiments.

Acknowledgements We are grateful to Dr B. Le Bossé for collecting plant material and for botanical identification, to Dr P. Guénot (CRMPO, Rennes, France) and L. Le Pollès for the mass measurement, to Dr S. Sinbandhit (CRMPO, Rennes, France) for advice on NMR, and to A. Bondon for performing HMBC spectra.

References and Notes

1) Part of this work was published as a poster during the ASP, AFERP, GA and PSE joint meeting "Past, Present and Future of Natural Products," hold in Amsterdam, 26—30th of July, 1999.
2) Bosser J., "La flore des Mascareignes," Vol. 109, Orstom, Paris, 1980.
3) Robin V., Boustie J., Amoros A., Girre L., *Pharm. Pharmacol. Commun.*, **4**, 61—64 (1998).
4) Batirov E. K., Matkarimov A. D., Malikov V., Yagudev M. R., *Khim. Prir. Soedin.*, **6**, 785—789 (1980).
5) Ngadjui B. T., Ayafor J. F., Sondengam B. L., Conolly J. D., *Phytochem.*, **28**, 585—589 (1989).
6) Breitmaier E., "Carbon 13 NMR Spectroscopy: High Resolution Methods and Application in Organic Chemistry and Biochemistry," ed. by Breitmaier E., Voelter W., VCH Publishers, New York, 1990.
7) Murray R. D. H., Mendez J., Brown S. A., "The Natural Coumarins," ed. by John Wiley & Sons Ltd., Wiley-Interscience Publication, New York, 1982.
8) Vilegas W., Boralle N., Cabrera A., Bernardi A. C., Pozetti G. L., Arantes S. F., *Phytochemistry*, **38**, 1017—1019 (1995).
9) Murray R. D. H., "Progress in the Chemistry of Organic Natural Products," Vol. 58, ed. by Herz W., Kirby G. W., Steglich W., Tamm Ch., Springer, New York, 1991, p. 343.
10) Wang Y., Hamburger M., Hostettmann K., *Planta Med.*, **55**, 109 (1989).
11) Wang Y., Hamburger M., Gueho J., Hostettmann K., *Phytochemistry*, **28**, 2323—2327 (1989).
12) Wang Y., Hostettmann K., *Bull. Liaison Groupe Polyphenols*, **15**, 167—170 (1990).
13) Xie L., Takeuchi Y., Cosentino L. M., Lee K. H., *J. Med. Chem.*, **42**, 2662—2672 (1999).
14) Huang L., Kashiwada Y., Cosentino L. M., Fan S., McPhail A. T., Fujioka T., Mihashi K., Lee K., *J. Med. Chem.*, **37**, 3947—3955 (1994).
15) Cordell G. A., Kinghorn D., Pezzuto J. M., "Bioactive Natural Products," CRC Press, Boca Raton, 1993, pp. 195—216.
16) Denizot L., Lang R., *J. Immunol. Methods*, **89**, 271—277 (1986).
17) Pauwels R., Balzarini J., Baa M., Snoeck R., Herdewijn P., Desmyter J., De Clercq E., *J. Virol. Methods*, **20**, 309—321 (1988).
18) Carmichael J., Degraff W. G., Gezdar A.F., Ninna J. D., Mitchell J. B., *Cancer Res.*, **47**, 936—942 (1987).