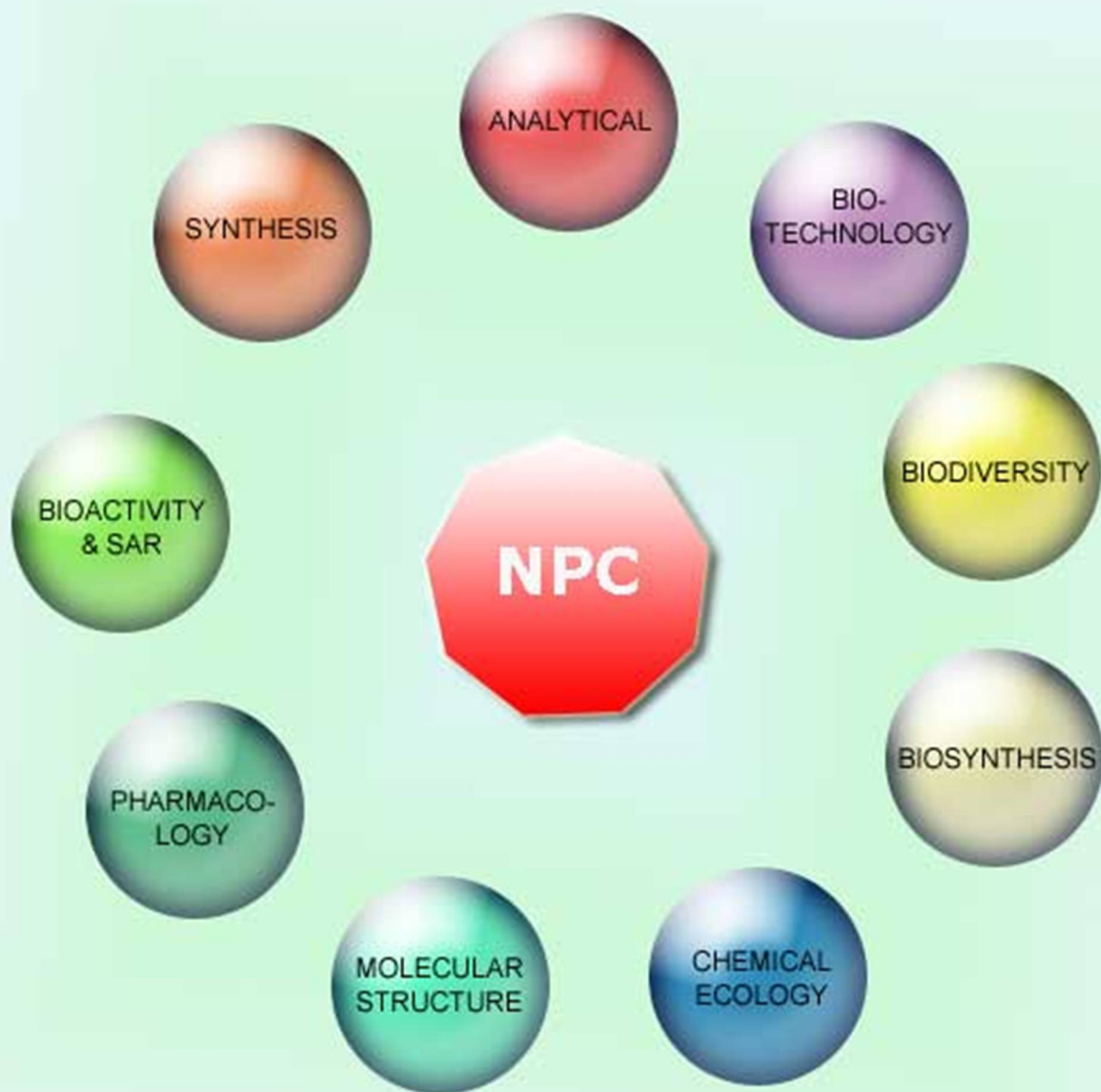


NATURAL PRODUCT COMMUNICATIONS

An International Journal for Communications and Reviews Covering all
Aspects of Natural Products Research



Volume 5. Issue 11. Pages 1711-1846. 2010
ISSN 1934-578X (printed); ISSN 1555-9475 (online)
www.naturalproduct.us

EDITOR-IN-CHIEF**DR. PAWAN K AGRAWAL**

Natural Product Inc.
7963, Anderson Park Lane,
Westerville, Ohio 43081, USA
agrawal@naturalproduct.us

EDITORS**PROFESSOR ALESSANDRA BRACA**

Dipartimento di Chimica Bioorganica e Biofarmacia,
Universita di Pisa,
via Bonanno 33, 56126 Pisa, Italy
braca@farm.unipi.it

PROFESSOR DEAN GUO

State Key Laboratory of Natural and Biomimetic Drugs,
School of Pharmaceutical Sciences,
Peking University,
Beijing 100083, China
gda5958@163.com

PROFESSOR J. ALBERTO MARCO

Departamento de Química Organica,
Universidade de Valencia,
E-46100 Burjassot, Valencia, Spain
alberto.marco@uv.es

PROFESSOR YOSHIIHIRO MIMAKI

School of Pharmacy,
Tokyo University of Pharmacy and Life Sciences,
Horinouchi 1432-1, Hachioji, Tokyo 192-0392, Japan
mimakiy@ps.toyaku.ac.jp

PROFESSOR STEPHEN G. PYNE

Department of Chemistry
University of Wollongong
Wollongong, New South Wales, 2522, Australia
spyne@uow.edu.au

PROFESSOR MANFRED G. REINECKE

Department of Chemistry,
Texas Christian University,
Forts Worth, TX 76129, USA
m.reinecke@tcu.edu

PROFESSOR WILLIAM N. SETZER

Department of Chemistry
The University of Alabama in Huntsville
Huntsville, AL 35809, USA
wsetzer@chemistry.uah.edu

PROFESSOR YASUHIRO TEZUKA

Institute of Natural Medicine
Institute of Natural Medicine, University of Toyama,
2630-Sugitani, Toyama 930-0194, Japan
tezuka@innm.u-toyama.ac.jp

PROFESSOR DAVID E. THURSTON

Department of Pharmaceutical and Biological Chemistry,
The School of Pharmacy,
University of London, 29-39 Brunswick Square,
London WC1N 1AX, UK
david.thurston@pharmacy.ac.uk

HONORARY EDITOR**PROFESSOR GERALD BLUNDEN**

The School of Pharmacy & Biomedical Sciences,
University of Portsmouth,
Portsmouth, PO1 2DT U.K.
axuf64@dsl.pipex.com

ADVISORY BOARD

Prof. Berhanu M. Abegaz
Gaborone, Botswana

Prof. Viqar Uddin Ahmad
Karachi, Pakistan

Prof. Øyvind M. Andersen
Bergen, Norway

Prof. Giovanni Appendino
Novara, Italy

Prof. Yoshinori Asakawa
Tokushima, Japan

Prof. Lee Banting
Portsmouth, U.K.

Prof. Julie Banerji
Kolkata, India

Prof. Anna R. Bilia
Florence, Italy

Prof. Maurizio Bruno
Palermo, Italy

Prof. Josep Coll
Barcelona, Spain

Prof. Geoffrey Cordell
Chicago, IL, USA

Prof. Cristina Gracia-Viguera
Murcia, Spain

Prof. Duvvuru Gunasekar
Tirupati, India

Prof. A.A. Leslie Gunatilaka
Tucson, AZ, USA

Prof. Kurt Hostettmann
Lausanne, Switzerland

Prof. Martin A. Iglesias Arteaga
Mexico, D. F., Mexico

Prof. Jerzy Jaroszewski
Copenhagen, Denmark

Prof. Leopold Jirovetz
Vienna, Austria

Prof. Teodoro Kaufman
Rosario, Argentina

Prof. Norbert De Kimpe
Gent, Belgium

Prof. Karsten Krohn
Paderborn, Germany

Prof. Hartmut Laatsch
Gottingen, Germany

Prof. Marie Lacaille-Dubois
Dijon, France

Prof. Shoen-Sheng Lee
Taipei, Taiwan

Prof. Francisco Macias
Cadiz, Spain

Prof. Imre Mathe
Szeged, Hungary

Prof. Joseph Michael
Johannesburg, South Africa

Prof. Ermino Murano
Trieste, Italy

Prof. M. Soledade C. Pedras
Saskatoon, Canada

Prof. Luc Pieters
Antwerp, Belgium

Prof. Om Prakash
Manhattan, KS, USA

Prof. Peter Proksch
Düsseldorf, Germany

Prof. Phila Raharivelomanana
Tahiti, French Polynesia

Prof. Satyajit Sarker
Wolverhampton, UK

Prof. Monique Simmonds
Richmond, UK

Prof. Valentin Stonik
Vladivostok, Russia

Prof. Winston F. Tinto
Barbados, West Indies

Prof. Karen Valant-Vetschera
Vienna, Austria

Prof. Peter G. Waterman
Lismore, Australia

INFORMATION FOR AUTHORS

Full details of how to submit a manuscript for publication in Natural Product Communications are given in Information for Authors on our Web site <http://www.naturalproduct.us>.

Authors may reproduce/republish portions of their published contribution without seeking permission from NPC, provided that any such republication is accompanied by an acknowledgment (original citation)-Reproduced by permission of Natural Product Communications. Any unauthorized reproduction, transmission or storage may result in either civil or criminal liability.

The publication of each of the articles contained herein is protected by copyright. Except as allowed under national "fair use" laws, copying is not permitted by any means or for any purpose, such as for distribution to any third party (whether by sale, loan, gift, or otherwise); as agent (express or implied) of any third party; for purposes of advertising or promotion; or to create collective or derivative works. Such permission requests, or other inquiries, should be addressed to the Natural Product Inc. (NPI). A photocopy license is available from the NPI for institutional subscribers that need to make multiple copies of single articles for internal study or research purposes.

To Subscribe: Natural Product Communications is a journal published monthly. 2010 subscription price: US\$1,695 (Print, ISSN# 1934-578X); US\$1,695 (Web edition, ISSN# 1555-9475); US\$2,095 (Print + single site online); US\$595 (Personal online). Orders should be addressed to Subscription Department, Natural Product Communications, Natural Product Inc., 7963 Anderson Park Lane, Westerville, Ohio 43081, USA. Subscriptions are renewed on an annual basis. Claims for nonreceipt of issues will be honored if made within three months of publication of the issue. All issues are dispatched by airmail throughout the world, excluding the USA and Canada.

New Acyclic Diterpenic Acids from Yacon (*Smallanthus sonchifolius*) Leaves

María I. Mercado^{a,b}, María V. Coll Aráoz^{a,b}, Alfredo Grau^b and César A. N. Catalán^{a*}.

^aINQUINOA-CONICET, Instituto de Química Orgánica, Facultad de Bioquímica Química y Farmacia, Universidad Nacional de Tucumán, Ayacucho 491, (T4000INI) San Miguel de Tucumán, Argentina

^bInstituto de Ecología Regional (IER), Facultad de Ciencias Naturales, Universidad Nacional de Tucumán, C.C. 34, (4107) Yerba Buena, Tucumán, Argentina

ccatalan@fbqf.unt.edu.ar

Received: June 18th, 2010; Accepted: August 18th, 2010

Two new acyclic diterpenoids, smaditerpenic acid E (**1a**) and F (**2a**), along with nineteen melampolide-type sesquiterpene lactones, six of them not previously reported in yacon, were isolated from the methylene chloride leaf rinse extract. Their structures were elucidated from 1D and 2D NMR experiments and gas chromatography coupled to mass spectrometry.

Keywords: *Smallanthus sonchifolius*, yacon, acyclic diterpenes, geranylnerol derivatives, sesquiterpene lactones, epicuticular wax.

Yacon, *Smallanthus sonchifolius* (Poepp. & Endl.) H. Robinson (Asteraceae), is a perennial herb from the Andean region of South America. Yacon tuberous roots are rich in fructooligosaccharides (FOS) and phenolic compounds, being consumed raw as 'fruits' since pre-Columbian times. In the past two decades yacon was introduced into several Asian and European countries. The Asian dispersal began in Japan, where the use of its leaves, emerged, apparently for the first time, as an anti-diabetic herbal tea. Potent antioxidant [1] and antidiabetic properties have been recently demonstrated for decoctions of yacon leaves [2-4] where enhydrin, its main sesquiterpene lactone, along with several caffeic acid derivatives have been shown to be the active principles [5,6]. Therefore, yacon has recently become popular as a healthy herbal tea. It should be noted that in spite of its Andean origin, no evidence has been found for its use in traditional Andean medicine [7].

Melampolide-type sesquiterpene lactones (STL) [8-11], acyclic diterpenic acids [12] and *ent*-kaur-16-en-19-oic acid (kaurenoic acid) derivatives [13-16] have been isolated from *S. sonchifolius* leaves. Several of these compounds play a physiological role in pest-resistance and antimicrobial activities [9,13]. The melampolide enhydrin, a major constituent of the leaf rinse extract, shows anti-inflammatory [17,18] and antidiabetic properties [5].

In this work we report two new acyclic diterpenes along with nineteen melampolide-type STL and many other constituents isolated from dichloromethane leaf rinse extracts. The two new diterpenes reported here, named smaditerpenic acids E (**1a**) and F (**2a**), are closely related to smaditerpenic acids A-D (**3-6**) (Figure 1) recently isolated from a Chinese collection of yacon extracted with boiling water [12]. Possible artifacts formation during this process is briefly discussed below. Interestingly, diterpenes **3-6** exhibited a moderate inhibitory effect on α -glucosidase [19]. Additionally, a rapid and sensitive GC-MS method for analysis of STL in leaf rinse extracts is described, which permits identification and quantification of minor constituents.

Smaditerpenic acids E (**1a**) and F (**2a**) and sesquiterpene lactone **11** co-eluted as a 1:1:1 mixture from column chromatography over Si gel of dewaxed extract (see experimental). The mixture was treated with diazomethane to yield the corresponding methyl esters **1b**, **2b** and **12** (sonchifolin), respectively, which were cleanly separated by RP-HPLC.

Smaditerpenic acid E methyl ester (**1b**) showed IR absorptions for hydroxyl, saturated and α,β -unsaturated ester groups at 3350, 1735 and 1715 cm^{-1} respectively. The molecular formula $\text{C}_{23}\text{H}_{36}\text{O}_5$, followed from its HR-CIMS, which showed a quasimolecular ion peak

Table 1: ^1H and ^{13}C NMR spectroscopic data of **1b** (300 MHz, CDCl_3).

Position	δ_{H} (J)	COSY	δ_{C} (DEPT)	HMBC	NOE
1	4.11 brd (2H, 7.1)	2, 20	59.0		4, 2
2	5.45 brt (1H, 7.5)	1, 20, 4	125.0	20	
3	-	-	138.9	1, 20	
4	2.14 brt (2H, 7.5)*		31.9	20	
5	2.24 brq (2H, 7.2)*		26.3		
6	5.42 brt (1H, 7.4)	19, 5	130.6	19, 8, 4	
7	-	-	133.7	19	
8	2.19 brt (2H, 7.1)*		34.8	10, 19	
9	2.54 brq (2H, 7.5)	8, 10	28.1		
10	5.84 brt (1H, 7.3)	9, 12	141.5	12	12, 9
11	-	-	131.9	9	
12	2.26 brt (2H, 7.5) *		34.7		
13	2.11 brq (2H, 7.1)*		27.8		
14	5.08 brt (1H, 7.0)	16, 17, 13	123.4	17, 16	16, 13
15	-	-	132.3	16, 17	
16	1.68 s (3H)	17, 13, 14	25.7	17	
17	1.58 s (3H)	16, 13, 14	17.7	16	
18	-	-	168.4	1'	
19	4.59 s (2H)	6, 5	61.7		9, 5
20	1.74 s (3H)	1, 2	23.4	4	
1'	3.73 s (3H)		51.2		
Ac	2.10 s (3H)		21.0		
-	-	-	171.1	19	

Assignments based on HSQC, COSY, HMBC and NOE experiments; coupling constants are in Hz. *Overlapped signals.

$[\text{M}+\text{H}]^+$ at m/z 393.2644 (calcd 393.2640) accounting for six degrees of unsaturation (Figure 1). The ^1H NMR spectrum of **1b** (Table 1) displayed four olefinic protons at δ 5.45 and 5.42 (two overlapped brt, 2H, 7.5 Hz, H-2 and H-6 respectively), 5.84 (brt, 1H, 7.3 Hz, H-10) and 5.08 (brt, 1H, 7.0 Hz, H-14). In addition, signals at δ 4.11 (brd, 2H, 7.1 Hz, 1-H) and 4.59 (s, 2H, H-19) showing correlation with signals at δ 59.0 and 61.7 in the HSQC spectrum indicated that two $-\text{CH}_2\text{O}-$ groups were present. Five methyl signals were also present at δ_{H} 3.73 (s, 3H, $-\text{CO}_2\text{Me}$), 2.10 (s, 3H, acetate), 1.74 (s, 3H, H-20), 1.68 (3H, s, H-16), and 1.58 (3H, s, H-17), which were correlated with signals at δ_{C} 51.2, 21.0, 23.4, 25.7, and 17.7, respectively, the overall spectrum clearly indicating that a geranyl nerol derivative containing a carboxymethyl group and an acetate residue was present. The deshielding of the protons at C-19 (δ_{H} 4.59) and their correlation with the acetate carbonyl at δ_{C} 171.1 in the HMBC experiment indicated that the acetate was located at C-19. Interpretation of the ^{13}C NMR, COSY, HSQC and HMBC spectra permitted assignment of all signals (Table 1). The configuration of the double bonds was deduced from NOE spectra.

The molecular formula $\text{C}_{24}\text{H}_{38}\text{O}_5$ was deduced from the HR-CIMS for smaditerpenic acid F methyl ester (**2b**), which showed a quasimolecular ion peak $[\text{M}+\text{H}]^+$ at m/z 407.2799 (calcd for $\text{C}_{24}\text{H}_{39}\text{O}_5$ 407.2797) accounting for six degrees of unsaturation (Figure 1). The ^1H - and ^{13}C -NMR spectra of **2b** (Table 2) were similar to those

Table 2: ^1H and ^{13}C NMR spectroscopic data of **2b** (300 MHz, CDCl_3).

Position	δ_{H} (J)	COSY	δ_{C} (DEPT)	HMBC	NOE
1	4.10 d (2H, 7.0)	2, 20	58.92		2, 4
2	5.45 brt (1H, 7.5)	1, 20	124.97	20	
3	-	-	138.84	1, 20	
4	2.14 m (2H)*		31.84	20	
5	2.19 m (2H)*		26.27		
6	5.43 brt (1H, 7.5)	5	130.63	19	
7	-	-	133.58	19, 5	
8	2.17 m (2H)*		34.76		
9	2.56 brq (2H, 7.5)	10, 8	28.08		
10	5.87 brt (1H, 7.5)	9	141.56	12	12, 9, 8
11	-	-	132.03	9	
12	2.37 dd (2H, 8.6, 7.3)	13	33.42		
13	2.12 m (2H)*		34.06		
14	-	-	155.06	16, 17	
15	2.22 m (1H)*	16, 17	33.76	21a, 21b, 16, 17	
16	} 1.01 d (6H, 6.8)	15	21.72	15, 17	21b, 15, 13
17				15, 16	
18	-	-	168.29	1'	
19	4.59 s (2H)	5, 6	61.69		5, 9
20	1.74 brs (3H)	1, 2	23.34	2, 4	
21a	4.67 brs (1H)	} 21b, 13	106.85	15, 13	
21b	4.76 brs (1H)				
1'	3.75 s (3H)		51.24		
Ac	2.06 s (3H)		20.97		
-	-	-	171.08	19	

Assignments based on HSQC, COSY, HMBC and NOE experiments; coupling constants are in Hz. *Overlapped signals.

of **1b** showing clearly that we were dealing with a homolog containing an additional $=\text{CH}_2$ group at C-14. Interpretation of the spectroscopic data permitted assignment of all signals and stereochemistry of **2b** (Table 2).

Smaditerpenic acids **E** (**1a**) and **F** (**2a**) are closely related to acyclic diterpenes **3-6** previously extracted from yacon leaves using water under reflux [12]. As can be seen in Figure 1, smaditerpenic acids **A** (**3**), **B** (**4**), **C** (**5**) and **D** (**6**) could be artifacts formed by hydrolysis of the acetate residue at C-19, followed by allylic rearrangement of the primary carbinol group at C-1 of **1a** and **2a**, respectively. To test this hypothesis, 20 mg of smaditerpenic acid **F** (**2a**) dissolved in 10 mL of water was refluxed for 24 h and extracted with chloroform. ^1H NMR analysis of the reaction product showed that very little rearrangement (ca. 4%) had occurred as deduced by integration of the signals corresponding to the vinyl moiety of **6** (Figure 1). Similar results were obtained when **1a** was subjected to the same treatment. These results suggest that smaditerpenic acids **A-D** (**3-6**) are also true natural products, which is supported by the chirality observed for **4** and **6** [12]. The difference between the diterpenes isolated by us and those previously reported by Chinese workers [12] could be due to agroecological growing conditions or most probably to a different cultivar (clone).

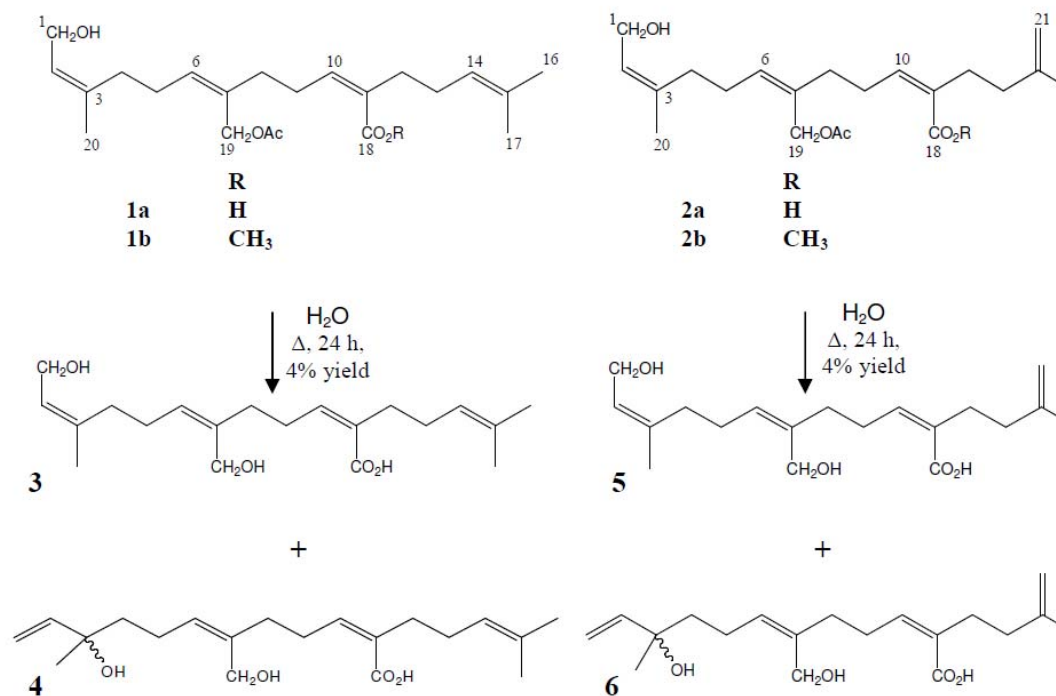
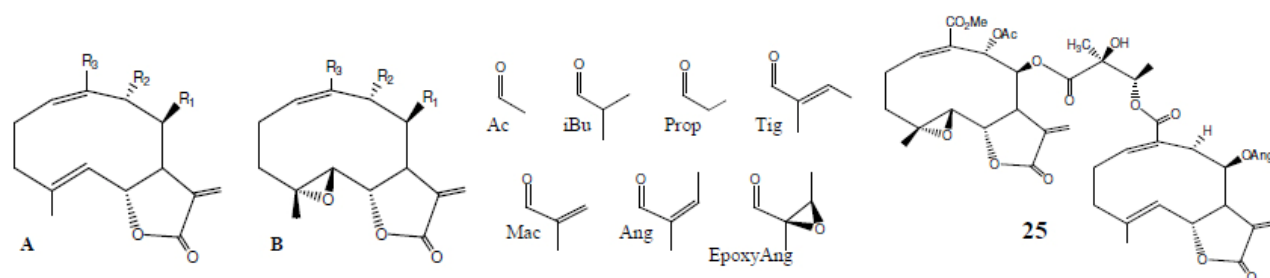


Figure 1: Smaditerpenic acids E (1a) and F (2a) and products (smaditerpenic acids A-D, 3-6) obtained after refluxing for 24 h with water.



Compound	Skeleton	R ₁	R ₂	R ₃	M+	RT	KI	RRI	%	
7	A	OProp	H	CO ₂ Me	348	25.93	2534	0.891	trace*	
8	A	OiBu	H	CO ₂ Me	362	26.88	2563	0.900	trace*	
9	A	OMac	H	CO ₂ Me	360	28.13	2609	0.916	trace*	
10	A	OMac	OAc	CO ₂ Me	418	29.73	2654	0.932	trace*	
11	A	OAng	H	CO ₂ H	360	31.71	2711	0.952	trace*	
12	Sonchifolin	A	OAng	H	CO ₂ Me	374	31.85	2716	0.953	1.2
13	A	OAng	H	CHO	344	32.34	2724	0.958	trace*	
14	A	OTig	H	CO ₂ Me	374	33.28	2761	0.969	trace*	
15	Polymatin B	A	OAng	OAc	CO ₂ Me	432	33.80	2775	0.974	5.5
16	A	OAng	OAc	CHO	402	34.30	2781	0.976	4.2	
17	Fluctuadin	B	OMac	OAc	CO ₂ Me	434	34.53	2788	0.978	4.0
18	Polymatin A	A	OAng	OH	CO ₂ Me	390				
19	A	OTig	OAc	CO ₂ Me	432	34.96	2800	0.983	trace*	
20	Uvedalin	A	OEpoxyAng	OAc	CO ₂ Me	448	36.45	2847	1.000	16.8
21	A	OEpoxyAng	OAc	CHO	418	36.65	2863	1.005	3.7	
22	Fluctuanin	B	OAng	OAc	CO ₂ Me	448	38.27	2914	1.023	8.4
23	Polymatin C	B	OAc	OEpoxyAng	CO ₂ Me	464	39.20	2942	1.033	5.6
24	Enhydrin	B	OEpoxyAng	OAc	CO ₂ Me	464	40.58	2977	1.045	48.7

Table 3: Melampolides isolated from leaf rinse extract of *Smallanthus sonchifolius*. *Trace <0.5%.

So far, sixteen melampolide-type STL have been identified in yacon leaves [8-11], enhydrin being the main lactone constituent. In this work we report a capillary GC-MS method to analyze STL from yacon which permitted quantification and identification of very minor and trace constituents (see experimental). Quantification is relevant since enhydrin (**24**) has recently been characterized as one of the antidiabetic principles of yacon [5]. Eighteen melampolides were identified in the dewaxed leaf rinse extract and are listed in Table 3. Enhydrin (**24**) is the main constituent representing almost a half of the total STL mixture, followed by uvedalin (**20**) (16.8%) and fluctuanin (**22**) (8.4%). Lactones **7**, **8**, **17** [20], **19**, **21** and **23** [21] are reported in yacon for the first time. Dimeric melampolide **25** [10], which is not volatile enough to be analyzed by GC, was isolated from the more polar fractions of the dewaxed extract.

Experimental

General experimental procedures: Melting points were determined on a Ernst Leitz 350 microscope. Infra-red spectra were recorded on a Perkin-Elmer 1600 FT-IR spectrophotometer. NMR measurements were recorded on a Bruker 300 AVANCE. Two dimensional homonuclear (^1H - ^1H COSY, ^1H - ^1H NOE) and heteronuclear experiments (^1H - ^{13}C $^1J_{\text{CH}}$ HSQC, ^1H - ^{13}C $^2\text{-}^3J_{\text{CH}}$ HMBC) were acquired and processed with TopSpin-NMR software provided by Bruker. For GC-MS analysis, a 5973 Hewlett-Packard selective mass detector (quadrupole) coupled to a Hewlett-Packard 6890 GC fitted with an Elite-5MS Perkin-Elmer column (5% phenylmethylsiloxane, 30 m \times 0.25 mm i.d. \times 0.25 μm film thickness); ionization energy 70 eV, was used. The following conditions were employed to analyze STL: injector, GC-MS interphase, ion source and selective mass detector temperatures were maintained at 220°C, 280°C, 230°C and 150°C, respectively; ionization energy, 70 eV; injection size: 1 μL (split mode 80:1); carrier gas, helium at a flow rate of 1.2 mL min^{-1} . The oven was programmed as follows: from 180°C to 300°C at 2°C min^{-1} and then held at 300°C for 10 min. Solvent delay 4 min. In order to be injected, the samples were dissolved in methylene chloride using 25 μL of solvent per mg of STL mixture. Percentages are reported as the means of at least 3 runs and were calculated from the TIC (Total Ion Chromatogram) by the computer. Waxes were analyzed using an injector temperature of 280°C and the following oven temperature program: from 140°C to 300°C at 2°C min^{-1} and then held at 300°C for 10 min; the remaining conditions were the same as for STL.

Merck Silica gel (230-400 mesh, ASTM) was used for column chromatography (CC). For thin layer

chromatography, TLC aluminum sheets of silica gel 90 F₂₅₄ from Merck were used. Detection was achieved by spraying the plates with *p*-anisaldehyde-sulfuric acid reagent followed by heating.

For separation of methylated diterpenes and STL mixtures, Gilson 322 HPLC equipment with a differential refractive index detector was used. The following semipreparative columns were employed: i) a Beckman Ultrasphere C8 (5 μm , 10 \times 250 mm) and ii) a Beckman Ultrasphere C18 (5 μm , 10 \times 250 mm).

Plant material: Leaves of *S. sonchifolius* (clone LIEY 97-1) were collected in August 2008 from experimental plots cultivated at the Centro Universitario Horco Molle, Tucumán, Argentina (26° 47'S, 65° 19'W, 547 m a.s.l.). Voucher specimens are deposited in the herbarium of Instituto Miguel Lillo, San Miguel de Tucumán, Tucumán, Argentina (LIL 607173).

Extraction and isolation of waxes, sesquiterpene

lactones and acyclic diterpenes: Leaves of yacon were extracted by soaking air-dried leaves in CH_2Cl_2 following the procedure described by Schorr *et al.* [10], with modifications. Whole air-dried leaves (200 g) were soaked individually in CH_2Cl_2 (2.35 L), and placed in a rectangular TLC developing tank 22 \times 23 \times 7 cm made of common glass. Each leaf was soaked in the solvent for 20 secs at room temperature with a continuous and gentle swinging motion using large chromium-plated steel forceps. The solution was filtered through a filter paper and the solvent evaporated to yield 2.47 g of crude residue, which was dissolved in MeOH (35 mL) at 50°C to facilitate dissolution. After cooling, distilled water (15 mL) was added dropwise to precipitate waxes. Epicuticular waxes were concentrated in this precipitate. After filtering and drying in a vacuum dessicator, 1.15 g of waxes was obtained. The hydromethanolic filtrate was evaporated at reduced pressure to yield 1.25 g of dewaxed extract containing sesquiterpene lactones and diterpenes.

Waxes (1.10 g) were column chromatographed over Si gel 230-400 mesh (45 g) using *n*-hexane with increasing amounts of EtOAc (0-20%); 25 fractions were collected, which were analyzed by capillary GC-MS. The following compounds were identified: neophytadiene, γ -tocopherol, 6,10,14-trimethyl-2-pentadecanone, *n*-alkanes within the C₂₅-C₃₁ range (odd carbons predominating), 1-tetracosanol, 1-hexacosanol, 1-octacosanol, α - and β -amyrin, the phytosterols sitosterol (major) and stigmasterol (minor) and the diterpene *ent*-kaur-16-en-19-oic acid (kaurenoic acid). These compounds were characterized by comparison of their MS with those in the computer and NIST98

GC-MS libraries, and by co-injection with authentic samples, whenever available.

The dewaxed extract (1.25 g) was column chromatographed over Si gel (50 g; 230-400 mesh) using CHCl_3 with increasing amounts of EtOAc (10-40%); 152 fractions were collected, which were monitored and grouped into 17 fractions (A-Q) on the basis of their TLC profiles. After solvent evaporation, all the fractions were analyzed by FT-IR, GC-MS and ^1H NMR.

Fractions B (9 mg), C (39 mg), D (135 mg), E (49 mg), F (63 mg), G (93 mg), H (109 mg) and I (110) showing a γ -lactone carbonyl absorption at 1755-1780 cm^{-1} in their IR spectra were analyzed by GC-MS. Lactones **7**, **8**, **10**, sonchifolin (**12**) and polymatin B (**15**) were identified in fraction B; lactones **8**, **9**, **10**, **12**, **14** and **15** were identified in Fraction C; lactones **12**, **13**, **14**, **15**, **16**, **19**, uvedalin (**20**) and fluctuanin (**22**) were present in fraction D; **16**, **20** and **22** in fraction E; **20**, **21**, **22**, polymatin C (**23**) and enhydrin (**24**) in fractions E and F; and fluctuadin (**17**), polymatin A (**18**), **21**, **23** and **24** in fractions H and I. Analytical samples of the lactones were obtained by semipreparative RP-HPLC of the above fractions using a C8 (octyl) column with MeOH- H_2O 60 : 40 at a flow rate of 2.0 mL min^{-1} (injection: 1.2 mL of a solution containing 4.1 mg of STL mixture per mL) and re-chromatography on a C18 (octadecyl) column with MeOH- H_2O (1 : 1) at 1.8 mL min^{-1} , if necessary. Thus, 165 mg of enhydrin (**24**), 54 mg of uvedalin (**20**), 34 mg of fluctuanin (**22**), 19 mg of polymatin B (**15**), 3.5 mg of sonchifolin (**12**), 10 mg of 8 β -epoxyangeloyloxy-9 α -acetyloxy-14-oxo-1(10)*E*,4*E*,11(13)-germacatrien-6 α , 12-olide (**21**) and small amounts of several minor lactones, all of them listed in Table 3, were isolated. The isolated lactones were identified by MS and ^1H , ^{13}C NMR, HMBC and HSQC spectroscopic data.

Fractions J (18 mg) and K (32 mg) did not show γ -lactone carbonyl absorption and yielded no identifiable material. Fraction L (119 mg) was washed several times with diethyl ether to afford a white solid that was crystallized from EtOH-EtOAc 2:1 to yield 20 mg of dimeric lactone **25** [10] as white crystals, mp 263-265 $^\circ\text{C}$. The ^1H and ^{13}C NMR spectra were identical to those reported [10].

References

- [1] Valentová K, Sersen F, Ulrichová J. (2005) Radical scavenging and anti-lipoperoxidative activities of *Smallanthus sonchifolius* leaf extracts. *Journal of Agricultural and Food Chemistry*, **53**, 5577-5582.
- [2] Aybar MJ, Sánchez Riera AN, Grau A, Sánchez SS. (2001) Hypoglycemic effect of the water extract of *Smallanthus sonchifolius* (yacon) leaves in normal and diabetic rats. *Journal of Ethnopharmacology*, **74**, 125-132.

Fractions M (26 mg), N (47 mg) and O (59 mg) from CC of dewaxed extract were complex mixtures and gave no identifiable material.

Fraction P (143 mg) showed the presence of a carboxyl group in the IR spectrum and was shown to be a mixture of **1a**, **2a** and **11** by ^1H and ^{13}C NMR. A portion of this fraction (70 mg) was dissolved in diethyl ether and treated with an ethereal solution of diazomethane. After solvent evaporation, the residue was processed by RP-HPLC (C8 column; MeOH- H_2O 7:3 at 2.0 mL min^{-1}) to afford sonchifolin (**12**) (5 mg), smaditerpenic acid E methyl ester (**1b**) (15 mg) and smaditerpenic acid F methyl ester (**2b**) (19 mg).

Fraction Q (144 mg) was shown to be a complex mixture of polar compounds and pigments which yielded no identifiable material.

Smaditerpenic acid E, methyl ester (**1b**)

Gum.

^1H and ^{13}C NMR: Table 1.

MS (EI, 70 eV): m/z (%): 314 (1) [$\text{M} - \text{AcOH}$, $-\text{H}_2\text{O}$] $^+$, 299 (1) [$314 - \text{CH}_3$] $^+$, 281 (1), 267 (3), 255 (4), 245 (8), 231 (2), 213 (6), 199 (7), 185 (18), 171 (8), 159 (19), 145 (20), 133 (33), 119 (39), 105 (42), 95 (20), 91 (43), 84 (14), 81 (31), 79 (30), 77 (15), 69 (100), 67 (26), 59 (11), 55 (29), 53 (21), 43 (62), 41 (85).

HR-CIMS (isobutane): calcd for [$\text{C}_{23}\text{H}_{36}\text{O}_5 + \text{H}$] $^+$ m/z 393.2640; found: 393.2644.

Smaditerpenic acid F methyl ester (**2b**)

Gum.

^1H and ^{13}C NMR: Table 2.

MS (EI, 70 eV): m/z (%): 328 (1) [$\text{M} - \text{AcOH}$, $-\text{H}_2\text{O}$] $^+$, 313 (1) [$328 - \text{CH}_3$] $^+$, 299 (1), 285 (3), 269 (6), 253 (4), 245 (5), 229 (4), 213 (6), 201 (9), 196 (9), 185 (17), 168 (11), 159 (30), 151 (25), 145 (25), 133 (47), 119 (44), 107 (41), 105 (62), 93 (69), 91 (66), 81 (38), 79 (50), 67 (37), 55 (76), 43 (100), 41 (70).

HR-CIMS (isobutane): calcd for [$\text{C}_{24}\text{H}_{38}\text{O}_5 + \text{H}$] $^+$ m/z 407.2797; found: 407.2799.

Acknowledgments - Work in Argentina was supported by grants from Consejo Nacional de Investigaciones Científicas y Técnicas de Argentina (CONICET-PIP 00225) and Consejo de Investigaciones de la Universidad Nacional de Tucumán (CIUNT, 26/D416).

- [3] Baroni S, Suzuki-Kemmelmeier F, Caparroz-Assef SM, Cuman RKN, Bersani-Amado CA. (2008) Effect of crude extracts of leaves of *Smallanthus sonchifolius* (yacon) on glycemia in diabetic rats. *Brazilian Journal of Pharmaceutical Sciences*, **44**, 521-530.
- [4] Lachman J, Fernandez EC, Orsak M. (2003) Yacon [*Smallanthus sonchifolia* (Poepp. et Endl.) H. Robinson] chemical composition and use - a review. *Plant, Soil and Environment*, **49**, 283-290.
- [5] Genta SB, Cabrera WM, Mercado MI, Grau A, Catalan CA, Sanchez SS. (2010) Hypoglycemic activity of leaf organic extracts from *Smallanthus sonchifolius*: Constituents of the most active fractions. *Chemico-Biological Interactions*, **185**, 143-152.
- [6] Terada S, Itoh K, Noguchi N, Ishida T. (2009) Alpha-glucosidase for blood glucose level elevation and functional food containing tricaffeoylaldaric acid and method for producing tricaffeoylaldaric acid. United States Patent Application Publication, US 2009/0209649 A1.
- [7] Grau A, Rea J. (1997) Yacon. *Smallanthus sonchifolius* (Poepp. & Endl.) H. Robinson. In *Andean roots and tuber: Ahipa, Arracacha, Maca and Yacon. Promoting the conservation and use of underutilized crops*. Hermann M, Heller J (Eds). Institute of Plant Genetics and Crop Plant Research, Gatersleben/IPGRI, Rome, Italy.
- [8] Inoue A, Tamogami S, Kato H, Nakazato Y, Akiyama M, Kodama O, Akatsuka T, Hashidoko Y. (1995) Antifungal melampolides from leaf extracts of *Smallanthus sonchifolius*. *Phytochemistry*, **39**, 845-848.
- [9] Lin F, Hasegawa M, Kodama O. (2003) Purification and identification of antimicrobial sesquiterpene lactones from yacon (*Smallanthus sonchifolius*) leaves. *Bioscience, Biotechnology and Biochemistry*, **67**, 2154-2159.
- [10] Schorr K, Merford I, Da Costa FB. (2007) A novel dimeric melampolide and further terpenoids from *Smallanthus sonchifolius* (Asteraceae) and the inhibition of the transcription factor NF- κ B. *Natural Product Communications*, **2**, 367-374.
- [11] Hong SS, Lee SA, Han XH, Lee MH, Hwang JS, Park JS, Oh KW, Han K, Lee MK, Lee H, Kim W, Lee D, Hwang BY. (2008) Melampolides from the leaves of *Smallanthus sonchifolius* and their inhibitory activity of LPS-induced nitric oxide production. *Chemical & Pharmaceutical Bulletin*, **56**, 199-202.
- [12] Dou DQ, Tian F, Qiu YK, Xiang Z, Xu BX, Kang TG, Dong F. (2010) Studies on chemical constituents of the leaves of *Smallanthus sonchifolius* (yacon): Structures of two new diterpenes. *Natural Product Research*, **24**, 40-47.
- [13] Kakuta H, Seki T, Hashidoko Y, Mizutani J. (1992) Ent-kaurenic acid and its related compounds from glandular trichome exudate and leaf extracts of *Polymnia sonchifolia*. *Bioscience, Biotechnology and Biochemistry*, **56**, 1562-1564.
- [14] Dou DQ, Tian F, Qiu YK, Zheng X, Bi XX, Kang TG, Dong F. (2010) Studies on chemical constituents of the leaves of *Smallanthus sonchifolius* (yacon): Structures of two new diterpenes. *Natural Product Research*, **24**, 40-47.
- [15] Qiu YK, Kang TG, Dou DQ, Liang L, Dong F. (2008) Three novel compounds from the leaves of *Smallanthus sonchifolius*. *Journal of Asian Natural Products Research*, **10**, 1109-1115.
- [16] Ragasa CY, Alimboyoguen AB, Urban S, Raga DD. (2008) A bioactive diterpene from *Smallanthus sonchifolius*. *Natural Product Communications*, **3**, 1663-1666.
- [17] Feltenstein MW, Schühly W, Warnick JE, Fischer NH, Sufka KJ. (2004) Anti-inflammatory and anti-hyperalgesic effects of sesquiterpene lactones from Magnolia and Bear's foot. *Pharmacology Biochemistry and Behaviour*, **79**, 299-302.
- [18] Hwang D, Fischer NH, Jang BC, Tak H, Kim JK, Lee W. (1996) Inhibition of the expression of inducible cyclooxygenase and proinflammatory cytokines by sesquiterpene lactones in macrophages correlates with the inhibition of MAP kinases. *Biochemical and Biophysical Research Communications*, **226**, 810-818.
- [19] Zheng X, He F, Kang TG, Dou DQ, Gai K, Shi YY, Kim YH, Dong F. (2010) Anti-diabetes constituents in leaves of *Smallanthus sonchifolius*. *Natural Product Communications*, **5**, 95-98.
- [20] Ali E, Ghosh DPP, Pakrashi SC, Durham LJ, Duffield AM. (1972) Studies on Indian medicinal plants-XXVIII : Sesquiterpene lactones of *Enhydra fluctuans* Lour. Structures of enhydrin, fluctuanin and fluctuadin. *Tetrahedron*, **28**, 2285-2298.
- [21] Le Van N, Fischer NH. (1979) Three new melampolide sesquiterpenes, polymatin A, B and C, from *Polymnia maculata* Cav. var. *maculata*. *Phytochemistry*, **18**, 851-854.

A New Biisoflavonoid from the Roots of <i>Erythrina variegata</i> Hitoshi Tanaka, Masaru Sudo, Miyuki Hirata, Hideo Etoh, Masaru Sato, Ryoza Yamaguchi, Eiji Sakai, Ih-Sheng Chen and Toshio Fukai	1781
Chemical Constituents of <i>Nepeta distans</i> Javid Hussain, Nausheen Bukhari, Hidayat Hussain, Najeeb U Rehman and Syed Murtaza Hussain	1785
Two new Diarylheptanoids from <i>Alnus nitida</i> Imran N. Siddiqui, Viqar U. Ahmad, Aqib Zahoor, Amir Ahmed, Saleha S. Khan, Afsar Khan and Zahid Hassan	1787
(-)-Sclerotiorin from an Unidentified Marine Fungus as an Anti-meiotic and Anti-fungal Agent Li Bao, Zhenyu Xu, Shu-bin Niu, Michio Namikoshi, Hisayoshi Kobayashi and Hong-wei Liu	1789
Mitregenin, a New Annonaceous Acetogenin from <i>Mitrephora maingayi</i> Qiang Zhang, Ying-Tong Di, Hong-Ping He, Shun-Lin Li and Xiao-Jiang Hao	1793
Pycnanngloside: A New Cerebroside from Bark of <i>Pycnanthus angolensis</i> Valérie Béatrice Tsaassi, Hidayat Hussain, H�el�ene Tamboue, Etienne Dongo, Simeon F. Kouam and Karsten Krohn	1795
Long Argan Fruit Drying Time is Detrimental for Argan Oil Quality Hicham Harhar, Sa�id Gharby, Badr Eddine Kartah, Hanae El Monfalouti, Zoubida Charrouf and Dom Guillaume	1799
Volatiles from Steam-distilled Leaves of Some Plant Species from Madagascar and New Zealand and Evaluation of Their Biological Activity Rosaria Costa, Francesco Pizzimenti, Francesca Marotta, P. Dugo, Luca Santi and Luigi Mondello	1803
Volatile Constituents of Different Parts of <i>Smyrniolum olusatrum</i> from Greece Fotini Papaioannou, Aikaterini Koutsaviti and Olga Tzakou	1809
Volatile Constituents of <i>Senecio pterophorus</i> (African Daisy) DC. from South Africa Oladipupo A. Lawal and Adebola O. Oyedeji	1811
Essential Oil Constituents and Biological Activities of <i>Peristrophe bicalyculata</i> and <i>Borreria verticillata</i> Isiaka A. Ogunwande, Tameka M. Walker, Anita Bansal, William N. Setzer and Emmanuel E. Essien	1815
Insecticidal Activity Against <i>Bemisia tabaci</i> Biotype B of Peel Essential Oil of <i>Citrus sinensis</i> var. pear and <i>Citrus aurantium</i> Cultivated in Northeast Brazil Nicolle de Carvalho Ribeiro, Claudio Augusto Gomes da Camara, Fl�avia de Souza Born and Herbert �lvaro Abreu de Siqueira	1819
Composition and Antimicrobial Activity of the Leaf and Twig Oils of <i>Litsea mushaensis</i> and <i>L. linii</i> from Taiwan Chen-Lung Ho, Eugene I-Chen Wang, Yen-Hsueh Tseng, Pei-Chun Liao, Chien-Nan Lin, Ju-Ching Chou and Yu-Chang Su	1823
Essential Oil of <i>Turnera ulmifolia</i> Leaves from Cuba Jorge A. Pino	1829
Essential Oil of <i>Galinsoga parviflora</i> Leaves from Colombia Jorge A. Pino, Mauricio Gaviria, Juana Quevedo-Vega, Laura Garc�a-Lesmes and Clara E. Quijano-Celis	1831
Essential Oil Composition of Three Australian Endemic Species of <i>Darwinia</i> (Myrtaceae) Joseph J. Brophy, Robert J. Goldsack, Jes�s Pal�-Pa�l, Lachlan M. Copeland and Erich V. Lassak	1833
Chemistry and Biological Activity of Essential Oils from <i>Piper clausenianum</i> (Piperaceae) Andr� M. Marques, Anna L�a S. Barreto, Eber M. Batista, Jos� Alexandre da R. Curvelo, Leosvaldo S. M. Velozo, Davyson de L. Moreira, Elsie F. Guimar�es, Ros�ngela Maria A. Soares and Maria Auxiliadora C. Kaplan	1837
Antioxidant Activity and Chemical Composition of Essential Oil from <i>Atriplex undulata</i> Silvana A. Rodriguez and Ana P. Murray	1841

Natural Product Communications

2010

Volume 5, Number 11

Contents

<u>Original Paper</u>	<u>Page</u>
Alkaline Phosphatase (ALP) Enhancing Iridoid Glucosides from the Indonesian Medicinal Plant <i>Barleria lupulina</i> Retno Widjowati, Yasuhiro Tezuka, Tatsuro Miyahara, Suresh Awale and Shigetoshi Kadota	1711
Two New Sesquiterpenes from <i>Sarcandra glabra</i> Do Thi Oanh, Pham Thanh Ky, Nguyen Thi Bich Hang, Pham Hai Yen, Tran Hong Hanh, Nguyen Xuan Cuong, Dang Vu Luong, Chau Van Minh and Phan Van Kiem	1717
New Acyclic Diterpenic Acids from <i>Yacon (Smilax sonchifolius)</i> Leaves María I. Mercado, María V. Coll Aráoz, Alfredo Grau and César A. N. Catalán	1721
4-Deacetylbaconin III: a Proposed Biosynthetic Precursor of Paclitaxel from the Bark of <i>Taxus wallichiana</i> Muhammad Nisar, Mughal Qayum, Achyut Adhikari, Inamullah Khan, Waqar Ahamad Kaleem, Zulfiqar Ali and M. Iqbal Choudhary	1727
Chemical Composition of Natural Colophony from <i>Pinus brutia</i> and Comparison with Synthetic Colophony Ahmet C. Gören, Gökhan Bilsel, Alp Hakan Öztürk and Gülaçtı Topçu	1729
Straightforward Approach to the Discrimination of (4R)- and (4S)-β-Isocryptoxanthin from a Conformationally Insensitive CD Band Shinzo Hosoi, Takeyuki Tanaka, Yukiteru Katsumoto, Takashi Maoka, Toshio Fujiwara, Masayuki Yamashita and Manabu Node	1733
Two New Steroidal Saponins, Hylodoside A and Novaeguinoside Y, from the Starfish <i>Leptasterias hylodes reticulata</i> and <i>Culcita novaeguineae</i> (Juvenile) Eleonora V. Levina, Anatoly I. Kalinovskiy, Pavel S. Dmitrenok, Ekaterina A. Martyyas and Valentin A. Stonik	1737
New Steroidal Alkaloids from <i>Solanum hypomalacophyllum</i> Alida Pérez Colmenares, Libia Alarcón, Luis B. Rojas, Anne-Claire Mitaine-Offer, Laurent Pouységou, Stéphane Quideau, Thomas Paululat, Alfredo Usubillaga and Marie-Aleth Lacaille-Dubois	1743
Alkaloidal Constituents of <i>Tinospora crispa</i> M. Iqbal Choudhary, Muhammad Ismail, Zulfiqar Ali, Khozirah Shaari, Nordin H. Lajis and Atta-ur-Rahman	1747
Acetylcholinesterase and Butyrylcholinesterase Inhibitory Compounds from <i>Chelidonium majus</i> (Papaveraceae) Lucie Čahlíková, Lubomír Opletal, Milan Kurfürst, Kateřina Macáková, Andrea Kulhánková and Anna Hošťálková	1751
Identification of <i>Glycyrrhiza</i> Species by Direct Analysis in Real Time Mass Spectrometry Eriko Fukuda, Masaki Baba, Noriaki Iwasaki, Yoshihiro Uesawa, Kazunori Arifuku, Osamu Kamo, Koji Tsubono and Yoshihito Okada	1755
DPPH-Scavenging Activities and Structure-Activity Relationships of Phenolic Compounds Cheng-Dong Zheng, Gang Li, Hu-Qiang Li, Xiao-Jing Xu, Jin-Ming Gao and An-Ling Zhang	1759
RP-HPLC Analysis of <i>Jirakadyarishta</i> and Chemical Changes during Fermentation Uma Ranjan Lal, Shailendra Mani Tripathi, Sanjay M. Jachak, Kamlesh Kumar Bhutani and Inder Pal Singh	1767
Isoflavones from the Mangrove Endophytic Fungus <i>Fusarium</i> sp. (ZZF41) Zhongjing Huang, Jianxiang Yang, Zhigang She and Yongcheng Lin	1771
HPLC/DAD/MS and Antioxidant Activity of Isoflavone-Based Food Supplements Annalisa Romani, Pamela Vignolini, Annalisa Tanini, Barbara Pampaloni and Daniela Heimler	1775

Continued inside backcover