NPC Natural Product Communications

2006
Vol. 1
No. 4
281 - 285

Guaianolides from the Aerial Parts of *Centaurea hololeuca*

Sergio Rosselli[a], Antonella Maria Maggio[a], Rosa Angela Raccuglia[a], Monique S. J. Simmonds[b], Nelly A. Arnold[c] and Maurizio Bruno[a]*

[a]*Dipartimento Chimica Organica "E. Paternò", Università di Palermo, Viale delle Scienze, Parco d'Orleans II, 90128 Palermo, Italy*

[b]*Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, United Kingdom*

[c]*Università du Saint Esprit, Facultè de Agronomie, Kaslik, Beirut, Lebanon*

bruno@dicpm.unipa.it

Received: November 9th, 2005; Accepted: December 14th, 2005

Seven guaianolides were isolated from the acetone extract of the aerial parts of *Centaurea hololeuca* Boiss. The antifeedant activity of the natural compounds (**1**-**7**) and of four chloro derivatives (**8**-**11**), synthesized from repin (**1**) and janerin (**3**) were tested against larvae of *Spodoptera littoralis*. Cebellin J (**6**) and chlorojanerin (**11**) showed significant antifeedant activity at 100 ppm, whereas at this concentration cebellin G (**4**) and 15-deschloro-15-hydroxychlorojanerin (**7**) stimulated feeding. Cebellin G (**4**) stimulated larvae of *S. littoralis* to feed at low concentration, but deterred feeding at high concentrations. The addition of chlorine to repin (**1**) resulted in an increase in antifeedant activity.

Keywords: *Centaurea hololeuca*, Asteraceae, guaianolides, chemosystematics, antifeedant activity.

The genus *Centaurea* L. (Asteraceae, tribe Cardueae, subtribe Centaureineae) comprises *ca.* 600 species distributed in Asia, Europe, North Africa and America [1-2].

Previous chemical studies indicate that sesquiterpene lactones are systematically important within the genus *Centaurea*. Other secondary metabolites present in plants of this taxon include triterpenes, steroids, hydrocarbons, polyacetylenes, flavonoids, anthocyanins, lignans and alkaloids [3]. As part of our ongoing chemical investigation of species of *Centaurea* from the Mediterranean area [4-7], we have examined the aerial parts of the hitherto unstudied *C. hololeuca*.

C. hololeuca Boiss. belongs to Sect XV Seridia D.C. [8]. It is a plant entirely appressed-canescent; 40-100 cm high; stem is leafy, simple, 1-headed; leaves are oblong lanceolate, undivided except the lowest, which have sometimes 1-2 lobes at base; lower leaves tapering into petiole, upper smaller, sessili, short-decurrent. Heads are ovate-conical, truncate at base; prickles of involucre very short; flowerets yellow; pappus as long as achene or longer. Flowering in July-August. Habitat: subalpine regions. Endemic in Lebanon near the cedars of Antilebanon (Hermon) [9-10].

The aerial parts of the plant were extracted with acetone and the extract, after repeated column chromatography on silica gel, yielded in order of increasing polarity the following guaianolides: repin (**1**), cynaropicrin (**2**), janerin (**3**), cebellin G (**4**), babylin B (**5**), cebellin J (**6**) and 15-deschloro-15-hydroxychlorojanerin (hydroxyjanerin) (**7**).

The major guaianolide, repin (**1**), isolated for the first time from *Acroptilon repens* [11], has been shown to exhibit a variety of biological activities. The ingestion of *A.(Centaurea) repens* by horses has been reported to cause a movement disorder simulating Parkinson's disease and nigrostriatal degeneration. These effects have been ascribed to the high neurotoxicity of repin, the principal sesquiterpene lactone present in this species [12-14]. Furthermore, repin showed potent activity against *Entamoeba histolytica* and *Trichomonas vaginalis* [15].

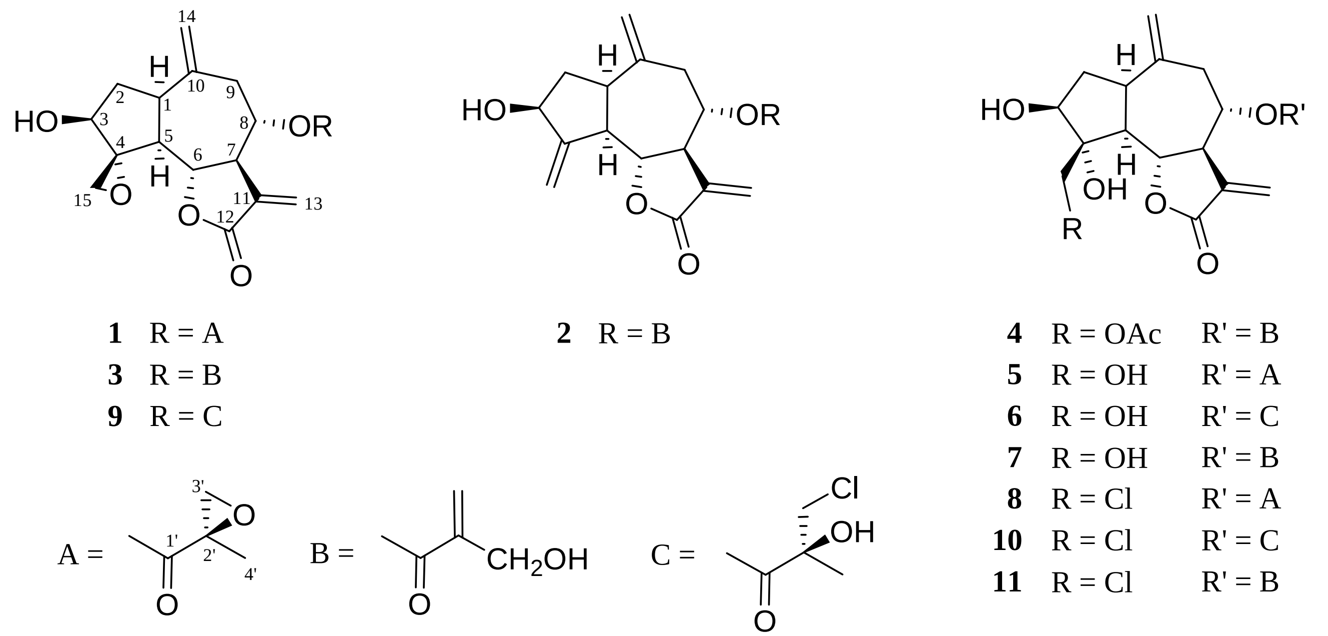

Figure 1. Structures of compounds **1-11**.

Various biological properties of other sesquiterpene lactones isolated from *C. hololeuca* have also been reported. Cynaropicrin (**2**), isolated for the first time from *Cynara scolymus* L. (artichoke) [16], has been the subject of many biological studies. For example, cynaropicrin has been shown to inhibit the proliferation of leukocyte cancer cell lines, such as U937, Eol-1 and Jurkat T cells, due to induction of apoptosis [17] and cytotoxicity against SK-OV-3, LOX-IMVI, A549, MCF-7, PC-3, and HCT-15 human cancer cell lines has been also reported [18-19]. Cynaropicrin also showed antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* [20], and trypanocidal activity against *Trypanosoma cruzi* [21]. It showed anti-inflammatory activity due to inhibition of tumor necrosis factor (TNF)-α and nitric oxide (NO) release [22], and was anti-hyperlipidemic [23].

Janerin (**3**), first found in *Centaurea janeri* Graells [24], has recently been shown to possess cytotoxic activity against human cancer cell lines of malignant melanoma (SK-MEL), epidermoid (KB), ductal (BT-549) and ovarian (SK-OV-3) carcinomas [25].

No biological activity has yet been reported for compounds **4-7** isolated, respectively, from *C. bella* [26], *C. babylonica* [27], *C. bella* [28] and *Saussurea candicans* [29]. Although several biological properties have been observed for sesquiterpene lactones, little is known about the influence of guaianolides on insect feeding behaviour [30]. We decided to investigate the antifeedant properties of compounds **1-11** against larvae of *Spodoptera littoralis* and evaluate the influence of chlorine atoms on the activity of the molecule. Four chloro-derivatives were synthesized. From repin (**1**), we synthesized the mono-chloro-derivatives solstitiolide (**8**) and chlorohyssopifolin C (**9**) and the di-chloro-derivative chlorohyssopifolin A (**10**). Similarly chlorojanerin (**11**) was prepared from janerin (**3**).

These four compounds (**8-11**), already known as natural products, were previously isolated from *Centaurea solstitialis* [31], *C. repens* [32], *C. hyssopifolia* [33], and *C. janeri* [24], respectively, and shown to possess biological properties. In fact, chlorojanerin (**11**) was identified as the main component responsible for the anti-ulcerogenic activity of the fresh spiny flowers extract of *C. solstitialis* L. ssp. *solstitialis* [34]. Chlorohyssopifolin A (**10**) has cytostatic activity against HeLa cells [35] and repin (**1**) and solstitiolide (**8**) have allelopathic properties [36]. Recently we reported on the cytotoxic activity of compounds **1**, **3**, **5**, **6, 9** and **10** against tumor cell replication. Repin (**1**), chlorohyssopifolin C (**9**) and chlorohyssopifolin A (**10**) showed significant antitumor potency [37].

Table 1: Effect of compounds **1-11** on the feeding behaviour of final stadium larvae of *S. littoralis*.

Compounds	FI[a]	FI_{50}[b]	FI_{-50}[c]
1	2.3±24.67	>1000	
2	-11.3±21.54	ndr	
3	17.5±19.06	ndr	
4	-47.3±3.19*	591	
5	3.3±15.82	ndr	
6	28.6±6.64*	ndr	
7	-50.9±10.99*		104
8	21.0±11.93	820	
9	39.8±13.62	ndr	
10	-21.6±6.55	>1000	
11	27.9±9.63*	ndr	

[a] FI = Feeding Index ((C-T)/(C+T))% at 100 ppm, * = p<0.05 Wilcoxon matched-pairs test.
[b/c] FI_{50} and FI_{-50} = concentration (ppm) calculated to give a Feeding Index of either 50% (antifeedant) or –50% (phagostimulant), respectively; ndr = no dose-dependent response.

The results from the feeding assay with larvae of *S. littoralis* are presented in Table 1. Cebellin J (**6)** and chlorojanerin (**11)** were the only two compounds to show significant antifeedant activity at 100 ppm, whereas cebellin G (**4**) and 15-deschloro-15-hydroxychlorojanerin (**7**) stimulated feeding at 100 ppm. As the concentration of cebellin G (**4)** increased it showed activity as an antifeedant and the concentration calculated to give an FI_{50} was 591 ppm. This marked dose-dependent change in activity was repeated in three different generations of larvae. 15-Deschloro-15-hydroxychlorojanerin (**7**) elicited a dose-dependent response as a phagostimulant and the concentration calculated to give a FI_{-50} was 104 ppm. The addition of hydrochloric acid to repin (**1**), as in solstitiolide (**8**) and chlorohyssopifolin C (**9**), resulted in an increase in antifeedant activity (Table 1), whereas the di-chloro- derivative, chlorohyssopifolin A (**10**), showed phagostimulant activity. When hydrochloric acid was added to janerin (**3**) the resulting compound, chlorojanerin (**11**), was significantly active as an antifeedant at 100 ppm. The results show that guaiane-type sesquitepene lactones can modulate the feeding behaviour of larvae of *S. littoralis* and justify further study.

Our chemical study of the aerial parts of *Centaurea hololeuca* has led to the isolation of seven guaiane-type sesquiterpene lactones (**1**-**7**), four of which, repin (**1**), janerin (**3**), babylin B (**5**) and cebellin J (**6**), were also identified as metabolites in *C. babylonica* L. [27] collected in the same area. This fact confirms the botanical assignment of these two species to the closely related sections Seridia (Juss.) DC, and Microlophus (Cass.) DC, respectively.

Experimental

General experimental procedures: Optical rotations were determined on a JASCO P-1010 digital polarimeter. ^{1}H and ^{13}C NMR, spectra were recorded on a Bruker AC 250 E MHz NMR spectrometer, using the residual solvent signal (δ = 7.27 in ^{1}H and δ = 77.00 in ^{13}C for $CDCl_3$ and δ = 2.05 in ^{1}H and δ = 30.50 in ^{13}C for acetone-d_6) as reference. ^{13}C NMR assignments were determined by DEPT spectra. ESI-MS was obtained with Applied Biosystem API-2000 mass spectrometer. Merck Art. 9025, 0.063-0.200 mm was used for silica gel column chromatography.

Dry aerial parts (1 kg), finely powdered, were extracted three times with acetone (3 x 10 L) at room temperature for one week. After filtration, the solvent was removed under reduced pressure to yield a residue (32 g), which was subjected to column chromatography (80 x 350 mm) and eluted with petroleum ether with increasing amounts of EtOAc, 500 mL fractions being collected as follows: 1-20 (petroleum ether), 21-38 (petroleum ether-EtOAc, 80/20), 39-43 (petroleum ether-EtOAc, 50/50), 44-50 (petroleum ether-EtOAc, 40/60), 51-55 (petroleum ether-EtOAc, 20/80), 56-80 (EtOAc), 81-86 (EtOAc-MeOH, 90/10).

Fractions 44-50 were rechromatographed by column chromatography, eluting with petroleum ether-EtOAc, (40/60) to give a subfraction that was allowed to crystallize (petroleum ether-EtOAc, 50/50) giving 500 mg of repin (**1**).

Fractions 51-55 were rechromatographed on a silica gel column, eluting with petroleum ether-EtOAc (50/50) to give two subfractions. The first one was further purified to give 265 mg of cynaropycrin (**2**) and the second, 350 mg of janerin (**3**).

Fractions 56-80 were rechromatographed on a silica gel column, using the same solvent system as described above to give two subfractions. The first one was allowed to crystallize (petroleum ether-EtOAc, 50/50) giving 14 mg of cebellin G (**4**).

Fractions 81-86 were rechromatographed to give 8 mg of babylin B (**5**), 9 mg of cebellin J (**6**) and 60 mg of 15-deschloro-15-hydroxychlorojanerin (**7**).

The structures of the isolated compounds were readily identified by comparing their physical and spectral data (melting points, optical rotation, NMR spectra, mass spectra) with those reported for repin (**1**), cynaropycrin (**2**), janerin (**3**), cebellin G (**4**), babylin B (**5**), cebellin J (**6**) and 15-deschloro-15-hydroxychlorojanerin (**7**).

Solstitiolide (8), chlorohyssopifolin C (9) and chlorohyssopifolin A (10): The synthesis of compounds **8-10** has been performed according to the procedures reported previously [37].

Chlorojanerin (11): Treatment of 15 mg of janerin (**3**) with 1.5 equiv. of lithium chloride and 1.5 equiv. of AcOH gave, after column chromatography (Si gel, 47/3 CH_2Cl_2-MeOH), 14 mg of chlorojanerin (**11**).

The physical and spectroscopic data were in perfect agreement with those reported in the literature [11, 16, 24, 26-29, 31-33].

Antifeedant bioassay: A binary choice bioassay using sucrose treated glass-fibre discs (Whatman 2.1 cm diameter) was used to investigate whether compounds influenced the feeding behaviour of final stadium larvae of Spodoptera littoralis (Lepidoptera) [38]. Larvae were placed singly in a Petri dish with a control disc (C) and a disc treated with the test compound (T). The respective amounts eaten of each disc were used to calculate the Feeding Index ((C-T)/(C+T))%. Antifeedant activity is represented by a positive value, whereas phagostimulant activity is represented by a negative value. The compounds were each tested at 4 to 5 concentrations (25 ppm, 50 ppm, 100 ppm, 250 ppm and 500 ppm). Each concentration was tested against from 8 to 25 different larvae. The Wilcoxon matched-pairs test was used to analyse the data. Regression analysis was used to calculate the concentration required to give a Feeding Index of 50% (FI_{50}) or –50% (FI_{-50}).

Acknowledgments - This work was supported by Italian Government project PRIN. The research at Kew on *Spodoptera littoralis* was covered by a Plant Health licence from defra.

References

[1] Hickey M, King CJ. (**1981**) *100 Families of Flowering Plants*. Cambridge University Press, New York.

[2] Heywood VH. (**1979**) *Flowering Plants of the World*. Oxford University Press.

[3] Al-Easa HS, Rizk AM. (**1992**) Constituents of *Centaurea* species. *Qatar University Science Journal*, ***12***, 27-57.

[4] Bruno M, Vassallo N, Fazio C, Gedris TE, Herz W. (**1998**) Sesquiterpene lactones of two *Centaurea* species from Sicily. *Biochemical Systematics and Ecology*, ***26***, 801-803.

[5] Bruno M, Maggio A, Paternostro MP, Rosselli S, Arnold NA, Herz W. (**2001**) Sesquiterpene lactones and other constituents of three Cardueae from Cyprus. *Biochemical Systematics and Ecology*, ***29***, 433-435.

[6] Bruno M, Maggio A, Rosselli S, Gedris TE, Herz W. (**2002**) Sesquiterpene lactones and other constituens of *Centaurea paniculata* ssp. *castellana*. *Biochemical Systematics and Ecology*, ***30***, 379-381.

[7] Senatore F, Rigano D, De Fusco R, Bruno M. (**2003**) Volatile components of *Centaurea cineraria* L. subsp. *umbrosa* (Lacaite) Pign. and *Centaurea napifolia* L. (Asteraceae), two species growing wild in Sicily. *Flavour and Fragrance Journal*, ***18***, 248-251.

[8] Boissier E. (**1875**) *Flora orientalis, Vol. **3***. Geneve et Basilee, 620.

[9] Post GE. (**1933**) *Flora of Syria, Palestine and Sinai, 2° Ed.* revised by J. E. Dinsmore, *Vol. **II***. American Press, Beirut, 117.

[10] Mouterde P. (**1983**) *Nouvelle Flora du Liban et de la Syrie, Tome III*. Darel-Marchreq, Beyrouth, 479.

[11] Evstratova RI, Rybalko KS, Sheichencko VI. (**1972**) The structure of the sesquiterpene repin. *Khimiya Prirodnykh Soedinenii*, 451-461.

[12] Robles M, Aregullin M, West J, Rodriguez E. (**1995**) Recent studies on the zoopharmacognosy, pharmacology and neurotoxicology of sesquiterpene lactones. *Planta Medica*, ***61***, 199-203.

[13] Robles M, Wang N, Kim R, Choi BH. (**1997**) Cytotoxic effects of repin, a principal sesquiterpene lactone of Russian knapweed. *Journal of Neuroscience Research*, ***47***, 90-97.

[14] Stevens KL, Riopelle RJ, Wong RY. (**1990**) Repin, a sesquiterpene lactone from *Acroptilon repens* possessing exceptional biological activity. *Journal of Natural Products*, ***53***, 218-221.

[15] Rubinchik MA, Rybalko KS, Evstratova RI, Konovalova OA. (**1976**) Sesquiterpene lactones of higher plants as a possible source of new antiprotozoal drugs. *Rastitel'nye Resursy*, ***12***, 170-181.

[16] Suchy M, Herout V, Sorm F. (**1960**) On terpenes. CXVI. Structure of cynaropicrin. *Collection of Czechoslovak Chemical Communications*, ***25***, 2777-2782.

[17] Cho JY, Kim AR, Jung JH, Chun T, Rhee MH, Yoo ES. (**2004**) Cytotoxic and pro-apoptotic activities of cynaropicrin, a sesquiterpene lactone, on the viability of leukocyte cancer cell lines. *European Journal of Pharmacology*, ***492***, 85-94.

[18] Li X, Qian P, Liu Z, Zhao Y, Xu G, Tao D, Zhao Q, Sun H. (**2005**) The sesquiterpenoids from *Cynara scolymus*. *Heterocycles*, ***65***, 287-291.

[19] Ha TJ, Jang DS, Lee JR, Lee KD, Lee J, Hwang SW, Jung HJ, Nam SH, Park KH, Yang MS. (**2003**) Cytotoxic effects of sesquiterpene lactones from the flowers of *Hemisteptia lyrata* B. *Archives of Pharmacal Research*, ***26***, 925-928.

[20] Modonova LD, Semenov AA, Zhapova T, Ivanova ND, Dzhaparova AK, Fedoseev AP, Kirdei EG, Malkova TI. (**1986**) Biological activity of extracts of *Saussurea amara*. *Khimiko-Farmatsevticheskii Zhurnal*, ***20***, 1472-1475.

[21] Schinor EC, Salvador MJ, Ito IY, De Albuquerque S, Dias DA. (**2004**) Trypanocidal and antimicrobial activities of *Moquinia kingii. Phytomedicine*, ***11***, 224-229.

[22] Cho JY, Baik KU, Jung JH, Park MH. (**2000**) In vitro anti-inflammatory effects of cynaropicrin, a sesquiterpene lactone, from *Saussurea lappa*. *European Journal of Pharmacology*, ***398***, 399-407.

[23] Shimoda H, Ninomiya K, Nishida N, Yoshino T, Morikawa T, Matsuda H, Yoshikawa M. (**2003**) Anti-hyperlipidemic sesquiterpenes and new sesquiterpene glycosides from the leaves of artichoke (*Cynara scolymus* L.): structure requirement and mode of action. *Bioorganic and Medicinal Chemistry Letters*, ***13***, 223-228.

[24] Gonzalez AG, Bermejo J, Cabrera I, Galindo A, Massanet GM. (**1977**) Quimica de las Compuestas XXIX. Principios activos de la *Centaurea janeri* Graells. *Anales de Quimica*, ***73***, 86-87.

[25] Muhammad I, Takamatsu S, Mossa JS, El-Feraly FS, Walker LA, Clark AM. (**2003**) Cytotoxic sesquiterpene lactones from *Centaurothamnus maximus* and *Vicoa pentanema*. *Phytotherapy Research*, ***17***, 168-173.

[26] Nowak G, Drozdz B, Holub M, Budesinsky M, S'Man D. (**1986**) Sesquiterpene lactones. XXXI. New guaianolides in *Centaurea bella* Trautv. and *Centaurea adjarica* Alb. *Acta Societatis Botanicorum Poloniae*, ***55***, 227-231.

[27] Bruno M, Rosselli S, Maggio A, Raccuglia RA, Arnold NA. (**2005**) Guaianolides from *Centaura babylonica*. *Biochemical Systematics and Ecology*, ***33***, 817-825.

[28] Nowak G, Holub M, Budesinsky M. (**1989**) Sesquiterpene lactones. XXXVI. Sesquiterpene lactones in several subgenera of the genus *Centaurea* L. *Acta Societatis Botanicorum Poloniae*, ***58***, 95-102.

[29] Singh P, Bhala M. (**1988**) Guaianolides from *Saussurea candicans*. *Phytochemistry*, ***27***, 1203-1205.

[30] Bhattacharyya PR, Barua NC, Ghosh AC. (**1995**) Cynaropicrin from *Trichlorepis glaberrima*: a potential insect feeding deterrent compound. *Industrial Crops and Products*, ***4***, 291-294.

[31] Merrill GB. Stevens KL. (**1985**) Sesquiterpene lactones from *Centaurea solstitialis*. *Phytochemistry*, ***24***, 2013-2018.

[32] Evstratova RI, Sheichenko VI, Rybalko KS. (**1973**) The structure of acroptilin – a sesquiterpene lactone from *Acroptilon repens*. *Khimiya Prirodnykh Soedinenii*, 161-167.

[33] Gonzalez AG, Bermejo J, Breton JL, Triana J. (**1972**) Constituents of Compositae. XV. Chlorohyssopifolin A and B, two new sesquiterpene lactones isolated from *Centaurea hyssopifolia* Vahl. *Tetrahedron Letters*, 2017-2020.

[34] Yesilada E, Gürbüz I, Bedir E, Tatli I, Khan IA. (**2004**) Isolation of anti-ulcerogenic sesquiterpene lactones from *Centaurea solstitialis* L. ssp. *solstitialis* through bioassay-guided fractionation procedures in rats. *Journal of Ethnopharmacology*, ***95***, 213-219.

[35] Gonzalez AG, Darias V, Alonso G, Estevez E. (**1980**) The cytostatic activity of the chlorohyssopifolins, chlorinated sesquiterpene lactones from *Centaurea*. *Planta Medica*, ***40***, 179-184.

[36] Stevens KL, Merrill GB. (**1985**) Sesquiterpene lactones and allelochemicals from *Centaurea* species. *ACS Symposium Series*, ***268*** (*The Chemistry of Allelopathy*), 83-98.

[37] Bruno M, Rosselli S, Maggio A, Raccuglia RA, Bastow KF, Lee K-H. (**2005**) Cytotoxic activity of natural and synthetic guaianolides. *Journal of Natural Products*, ***68***, 1042-1046.

[38] Simmonds MSJ, Blaney WM, Fellows LE. (**1990**) Behavioural and electrophysiological study of antifeedant mechanisms associated with polyhydroxyalkaloids. *Journal of Chemical Ecology*, ***16***, 3167-3196.