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Hypargyrin A, a Hemiacetalic Germacrolide from *Viguiera hypargyrea* (Asteraceae). Biogenetic Implications and Biological Evaluation

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Abstract. A new hemiacetalic germacrolide **6**, named hypargyrin A, was isolated from the aerial parts of *Viguiera hypargyrea*, along with the known compounds (8*R*,9*R*)-*β*-caryophyllene epoxide, *ent*-kaur-16-en-19-oic acid (**1**), hispidulin (**2**), 8*β*-epoxyangeloyloxy-14-acetoxy-tithifolin (**3**), 8*β*-epoxyangeloyloxy-14-hydroxy-tithifolin (**4**) and budlein B (**5**). Spectroscopic analysis of **6** allowed the structural correction of a compound isolated from *Blainvillea gayana*. Budlein B (**5**) can be considered the biogenetic precursor of **6**, and two pathways for its formation are proposed. Hypargyrin A (**6**) did not display significant anti-inflammatory activity against ear edema in mice induced by TPA, but showed cytotoxic activity against HeLa (IC_{50} 35.1 μM) and Hep-2 (39.2 μM) human cells.

Key words: Asteraceae, *Viguiera hypargyrea*, sesquiterpene lactones, germacrolides, hypargyrin A, budlein B, biogenesis, anti-inflammatory activity, cytotoxic activity.

Resumen. Una nueva germacrólida hemiacetálica **6**, denominada hypargirina, fue aislada de las partes aéreas de *Viguiera hypargyrea*, junto con los compuestos conocidos (8*R*,9*R*)-epóxido de *β*-cariofileno, ácido *ent*-kaur-16-en-19-oico (**1**), hispidulina (**2**), 8*β*-epoxiangeloiloxi-14-acetoxi-titifolina (**3**), 8*β*-epoxiangeloiloxi-14-hidroxi-titifolina (**4**) y budleína B (**5**). El análisis espectroscópico de **6** permitió la corrección estructural de un compuesto previamente aislado de *Blainvillea gayana*. La budleína B (**5**) puede considerarse como el precursor biogenético de **6** y se proponen dos rutas para su formación. La hypargirina A (**6**) no mostró actividad anti-inflamatoria significativa en el edema de rata inducido por el ATF, pero mostró toxicidad contra las líneas humanas celulares HeLa (IC_{50} 35.1 μM) y Hep-2 (IC_{50} 39.2 μM).

Palabras clave: Asteraceae, *Viguiera hypargyrea*, lactonas sesquiterpénicas, germacrólidas, hypargirina A, budleína B, biogénesis, actividad anti inflamatoria, actividad citotóxica

Introduction

The genus *Viguiera* is the largest within the subtribe Helianthinae of the family Asteraceae, comprising *ca*. 200 species. It is distributed mainly in the American Continent and adjacent islands, and it is divided in three subgenera (Amphilepsis, Calanticaria, Yerbalesia) which include several sections [1,2]. The section Hypargyrea include three species (*V. hypargyrea*, *V. decurrens* and *V. rosei*) and they are used in traditional medicine [1,3]. In Mexico the roots of *Viguiera hypargyrea* have been used for the treatment of stomach ailments and from this material were isolated friedelanes, oleanolic saponins [4] and some antispasmodic and antimicrobial diterpenes [5]. *Ent*-kauranes, sesquiterpenes, germacrolides [6] and flavonoids [7] have been previously isolated from the aerial part of this species.

As part of our search to discover bioactive naturally occurring compounds from plants [8], we report herein the structure of an additional germacrolide isolated from the aerial parts of *V. hypargyrea*, namely, hypargyrin A, along with six known compounds. Some comments on the biogenesis of this substance as well as its evaluation as anti-inflammatory and cytotoxic agent are also reported.

Results and Discussion

Chemical analysis of the aerial parts of *V. hypargyrea* afforded the known compounds (8*R*,9*R*)-*β*-caryophyllene epoxide [9], *ent*-kaur-16-en-19-oic acid (**1**) [10], hispidulin (**2**) [11] and the germacrolides 8*β*-epoxyangeloyloxy-14-acetoxy-tithifolin (**3**) [6,12], 8*β*-epoxyangeloyloxy-14-hydroxy-tithifolin (**4**) [6] and budlein B (**5**) [13] (Chart 1). In addition, compound **6** was isolated. The molecular formula of compound **6**, $C_{15}H_{18}O_5$, followed from its HREIMS. The IR absorptions indicated the occurrence of hydroxy group (3593 cm^{-1}) and *α*,*β*-unsaturated-*γ*-lactone (1764, 1666 cm^{-1}) functionalities. The ^{13}C NMR spectrum exhibited 15 signals which were assigned by the DEPT spectrum to the resonances of four quaternary carbons, six methines, four methylenes and one methyl carbon atoms, indicating the occurrence of a sesquiterpene lactone. Consequently, in the ^1H NMR spectrum the characteristic signals for the exocyclic vinylic protons appeared at δ_H 6.26 (H-13a) and 5.58 (H-13b), both being coupled with H-7 (δ_H 2.75). The observed allylic coupling ($J_{7,13a} = J_{7,13b} = 3.5$ Hz) indicated a *trans* configuration of the γ-lactone fused to a cyclodecadiene [14]. ^1H-^1H COSY experiment established the vicinity of the methine of the lactonic closure (δ_H 5.00, H-6) with the vinylic hydrogen (δ_H 5.23, H-5) and with H-7. This last signal was vicinally coupled with an oxygenated methine assigned to H-8 (δ_H 4.94; δ_C 72.16, C-8, assigned from the HSQC spectrum) which in turn was coupled with a methylene (H-9a and H-9b). The *β* orientation of the oxygen at C-8 was established by the observed NOESY correlation between H-7 and H-8, in agreement with the magnitude of the coupling constant ($J_{7,8}$ 1 Hz). The presence of the trisubstituted double bond at C(4)-C(5) was established by the signal of the vinylic

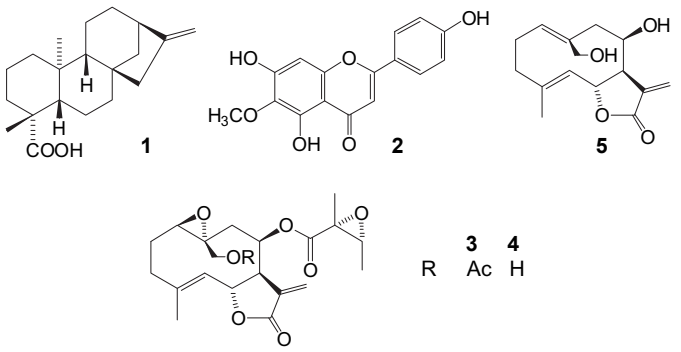


Chart 1.

methyl group in the ^{1}H NMR spectrum (δ_H 1.86, C-15) which was allylically coupled ($J_{5,15}$ 1 Hz) with H-5. The configuration of the endocyclic double bond was established as *trans* since the coupling between H-5 and H-6 ($J_{5,6}$ 10 Hz) was similar to that observed for other germacrolides [15]. Since C-10 is a quaternary oxygenated carbon (δ_C 68.89) which showed HMBC correlation with the oxymethine H-1 (δ_H 3.07; δ_C 67.16), an epoxide is located at C-1 and C-10. The remaining methine (δ_H 4.76) corresponded to H-14 and its carbon was linked to two oxygens (δ_C 93.76), establishing the C(8)-C(14) epoxide and the hydroxyl at C-14. These connectivities were confirmed from the HMBC correlations of H-1/C-14, H-1/C-9, H-14/C-10, H-14/C-8 and H-14/C-9.

Considering that the C-15 methyl group of the *trans*-configured endocyclic double bond is above the plane of the 10-membered ring (adopting a $^{15}D_5$ conformation), C-14 has to be *β*- oriented, to allow the hemiacetal formation with the hydroxyl at C-8*β*, thus defining the *R*- configuration at C-10. The structures of the two configurational possibilities at C-1 are shown in Figure 1 (**6** and **7**). The 1*S*,10*R* stereoisomer (structure **7,** epoxide derived from a melampolide in a $^1D^{14}$,$^{15}D_5$ conformation) has been proposed for a sesquiterpene lactone isolated from *Blainvillea gayana* [16], a plant belonging to a genus known to produce melampolides and germacrolides [17], and comparison of its physical and spectroscopic properties with those of the substance isolated from *Viguiera hypargyrea* indicated that these were the same substances. This finding was unexpected, since previous chemical studies have indicated that *Viguiera* species biosynthesize mainly germacrolides and heliangolides [2,18], but not melampolides. Herz proposed the 1*S*- configuration of **7** based on relative similarities of some coupling constants of relevant vicinal hydrogens with those of some melampolides, and on the relative downfield shift of H-8 (δ_H 4.94), attributed to the deshielding of the *a*- oriented hydroxyl at C-14 [16]. However, these arguments could be also considered relatively consistent with the epimeric structure **6**. NOESY experiments performed on the substance isolated from *V. hypargyrea* (Figure 1, **6A**) showed clear correlations between H-5 and H-1 and H-7, and between H-14 and H-15 and H-6; thus the configurations at C-1 and C-14 were 1*R*,14*R*, confirming structure **6** (epoxide derived from a [$_1D^{14}$, $^{15}D_5$] germacrolide) for the compound

Table 1. ^{1}H and ^{13}C NMR spectral data of hypargyryn (**6**)

Position	$^a\delta_H$ (J in Hz)	$^b\delta_C$
1	3.07 dd (11.0, 3.0)	67.16
2a	2.32 m	26.84
2b	1.28 m	
3a	2.36 m	35.74
3b	2.28 m	
4		145.47
5	5.23 dq (10.0, 1.0)	123.07
6*β*	5.00 dd (10.0, 10.0)	76.00
7*α*	2.75 dddd (10.0, 3.5, 3.5, 1.0)	56.10
8*α*	4.94 d (10.0, 1.0, 1.0)	72.16
9a	1.67 dd (13.5, 10.0)	36.41
9b	13.10 dd (13.5,1.0)	
10		68.89
11		138.18
12		169.35
13a	6.26 d (3.5)	118.52
13b	5.58 d (3.5)	
14*β*	4.76 s	93.76
15	1.86 d (1.0)	18.13

[a] 500 MHz, $CDCl_3$; [b] 125 MHz, $CDCl_3$

from *V. hypargyrea*, which adopts a chair-chair (crown) conformation. Therefore, the structure **7** for the compound isolated from *B. gayana* should be corrected to **6**. One and two dimensional NMR studies allowed to assign all the ^{1}H and ^{13}C NMR signals of **6** (see Table 1).

The biogenesis of **6** can be rationalized as shown in scheme **1**. Budlein B (**5**), also a natural constituent of *V. hypargyrea*, could be considered as its biogenetic precursor, and bio-epoxidation of the 1(10) double bond (pathway A) would afford intermediate **A** (8*β*,14-dihydroxy-tithifolin, following the trivial names for these compounds [6,19]), which in turn could be oxidized to the corresponding aldehyde (**B**), producing, after hemiacetalization, hypargyrin A (**6**). On the other hand, oxidation of the allylic alcohol of budlein A would afford the *α*,*β*-unsaturated aldehyde (**8**), which could undergo epoxidation to **B** (pathway B). Since the chemical transformation of **8** from **5** has been previously reported, and the germacrolide **8** undergoes acid catalyzed isomerization to

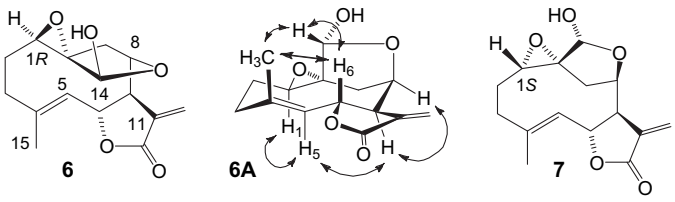
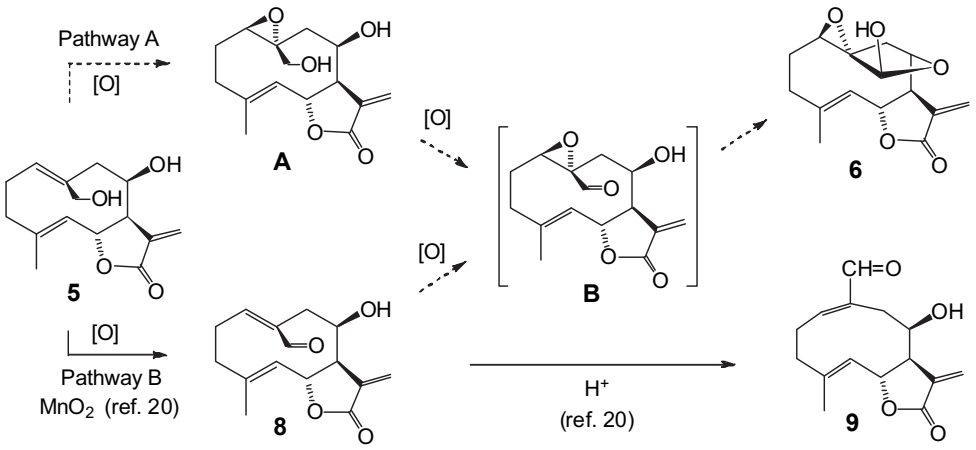


Fig. 1. Structures of hypargyrin (**6**) and **7**. Conformational representation of hypargyrin (**6A**) showing relevant NOESY correlations.



Scheme 1. Proposed terminal biogenesis of hypargyrin (**6**).

the melampolide *allo*-schkuhriolide (**9**) [20] (rather than hemiacetalization) [21-23], pathway A could be considered as the preferred biogenetic route for **6**. Although it is recognized the importance of the chemical reactivities of the natural products in their biosynthetic processes [24], the question whether the final biosynthetic steps of sesquiterpene lactones follow separate or simultaneous pathways remains open [25].

A broad variety of biological activities are known for sesquiterpene lactones, and they are believed to exert their action by alkylating biological macromolecules [26]. Their utility as therapeutic agents against inflammation and cancer has received considerable attention [27] and some constituents isolated from *Viguiera* species have recently been investigated [28].

The anti-inflammatory activity of hypargyrin A (**6**) against ear edema in mice induced by TPA was evaluated following reported procedures [29] and the results indicated that it displayed modest activity (18.4% of inhibition using a dose of 0.5 mg/ear) compared with the positive control indomethacin (66.9 % of inhibition at the same dose). The cytotoxicities of **6** against the HeLa (cervical), Hep-2 (larynx), K562 (leukemia), PC38 (prostate) and U251 (nervous system) human cell lines were also evaluated [30]. **6** displayed activity against HeLa (IC_{50} 35.1 ± 2.7 μM; positive control: 5-fluoro-uracil IC_{50} 1.5±0.19μM) and Hep-2 (39.2 ± 3.1 μM; positive control: 5-fluoro-uracil IC_{50}1.0±0.17 μM), showing no toxicity against the other cell lines.

Experimental

General Experimental Procedures. Uncorrected melting points were taken on a Fisher Johns apparatus. Optical rotations were determined on a Perkin Elmer 341 polarimeter and UV spectra on a Shimadzu UV 160 spectrophotometer. IR were recorded on a Nicolet Magna FT-IR 750 spectrometer. ^{1}H and ^{13}C spectra were measured on a Varian Unity Plus 500 spectrometer (at 500/125 MHz). EIMS were recorded on a Jeol JMS AX505HA spectrometer. Silica gel 60 230-400 and 70-230 (Merck) were used for column chromatography, and plates of Silica 60 GF 254 (Merck) were used for TLC.

Plant Material

The aerial parts (leaves, stems and flowers) of *Viguiera hypargyrea* Blake were collected 8 Km W of Durango City, Mexico, in September 15, 1997. A voucher specimen, identified by Dr. Robert Bye (acquisition MEXU 961417) has been deposited at the National Herbarium of Mexico, Instituto de Biología, Universidad Nacional Autónoma de México.

Extraction and Isolation

The dried and ground plant material (4 Kg) was extracted successively with hexane (15 L) at room temp (2 × 24 h) and with acetone (15 L) at room temp (3 × 48 h), to yield, after evaporation of the solvent under reduced pressure, 42 g and 90 g of extracts, respectively. The acetone extract was adsorbed on silica gel and chromatographed on a silica gel 60 (230-400) column (450 g) and eluted under vacuum with a hexane – ethyl acetate gradient solvent system, following the sequence 100:0, 95:5, 90:10; 85:15; 80:20; 75:25; 70:30; 65:35; 60:40; 50:50, 0:100. Fractions of 250 mL were collected. Some fractions eluted with hexane – ethyl acetate 95:5 were joined and rechromatographed on silica gel, obtaining a gum that was further purified by preparative TLC (eluting with hexane – ethyl acetate 9:1), to yield (8*R*,9*R*)-*β*-caryophyllene epoxide (14 mg) [9]. From subsequent fractions (eluted with hexane – ethyl acetate 95:5) was obtained a residue which was further purified by preparative TLC (developed with hexane – ethyl acetate, 4:1), to yield *ent*-kaur-16-en-19-oic acid (11 mg, **1**) [10]. Some fractions eluted with hexane – ethyl acetate 80:20 were joined and the residue (3 g) was purified using vacuum chromatography on silica gel, to obtain some subfractions which were joined and further purified by preparative TLC, to yield hispidulin (8 mg, **2**) [11]. Subsequent fractions of the main col-

umn (eluted with hexane – ethyl acetate 75:25) afforded a solid residue which was recrystallized from acetone – *iso*-propyl ether, to yield 8*β*-epoxyangeloyloxy-14-acetoxy-tithifolin (**3**), mp 212-214 ºC (acetone), lit: 213-214 ºC [6], 325 mg, R_f 0.43 (CH_2Cl_2 – acetone 95:5). From fractions eluted with hexane – ethyl acetate 7:3, a solid residue was obtained which was recrystallized from acetone – *iso*-propyl ether, to give 230 mg of 8*β*-epoxyangeloyloxy-14-hydroxy-tithifolin (**4**), mp 168-170 ºC (acetone), lit: 165-168 ºC [6], R_f 0.48 (CH_2Cl_2 – acetone 85:15). The 65:15 hexane – ethyl acetate fractions (1.9 g) of the main column were rechromatographed on silica gel with hexane –ethyl acetate gradient elution system, and crystallization of the solids obtained from some subfractions allowed to obtain 760 mg of budlein B (**5**), mp and mmp 162-163 ºC [13]. Several subfractions from this recromatography which showed the same spot on TLC were combined and the residue (150 mg) was purified by preparative TLC (developed with CH_2Cl_2 – acetone 7:3) to yield 10 mg of hypargyrin A (**6**). Mp 108-110 ºC (acetone), $[\alpha]_D^{25}$ – 2.2 ($CHCl_3$, 0.01), UV λ_{max} 212 nm (ε 7800), IR ($CHCl_3$) ν_{max} 3593, 1764, 1666, 955 cm^{-1}, ^1H and ^{13}C NMR (500 and 125 MHz, $CDCl_3$): see Table 1 (assignments by COSY, DEPT, HSQC, HMBC and NOESY); EIMS *m/z* (rel. int.): 278 (3), 260 (42), 240 (100), 211 (30), 151 (28), 71 (60), 43 (10). HREIMS *m/z* 279.1185 $[M + H]^+$, calcd for $C_{15}H_{18}O_5$ + H, 279.1188.

Biological Evaluations

The anti-inflammatory activity of hypargyrin A (**6**) was evaluated following the procedures previously reported [29], and its cytotoxic activities against HeLa (cervical cancer), Hep-2 (larynx cancer), K562 (leukemia), PC38 (prostate) and U251 (nervous system) cell lines were evaluated following standard procedures [30].

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References

1. Blake, S. F. *Contrib. Gray Herbarium Harvard Univ*. **1918**, *54*, 1-195.
2. a) Romo de Vivar, A.; Delgado, G. *Bol. Soc. Chil. Quím.* **1985**, *30*, 79-100. b) Ambrosio, S. R.; Tirapelli, C. R.; da Costa, F. B.; de Oliveira, A. M. *Life Sciences* **2006**, *79*, 925-933
3. Marquina, S.; Maldonado, N.; Garduño-Ramírez, M. L.; Aranda, E.; Villarreal, M. L.; Navarro, V.; Bye, R.; Delgado, G.; Álvarez, L. *Phytochemistry* **2001**, *56*, 93-97.
4. Álvarez, L.; Zamilpa, A.; Marquina, S.; González, M. *Rev. Soc. Quím. Méx*. **2003**, *47*, 173-177.
5. Zamilpa, A.; Tortoriello, J.; Navarro, V.; Delgado, G.; Alvarez, A. *Planta Medica* **2002**, *68*, 281-283.
6. a) Álvarez, L.; Mata, R.; Delgado, G.; Romo de Vivar, A. *Phytochemistry* **1985**, *24*, 2973-2976. b) Delgado, G.; Álvarez, L.; Soriano-García, M.; Toscano, R. A.; Mata, R.; Pereda-Miranda, R. *J. Nat. Prod.* **1987**, *50*, 273-276.
7. Wollenweber, E.; Doerr, M.; Roitman, J. N.; Schilling, E. *Zeit. Naturfor*. **1995**, *50*, 588-590.
8. León, A.; Reyes, B. M.; Chávez, M. I.; Toscano, R. A. Delgado, G. *J. Mex. Chem. Soc.* **2009**, *53*, 193-200.
9. Heymann, H.; Tezuka, Y.; Kikuchi, T.; Supritya tna, S. *Chem. Pharm. Bull.* **1994**, *42*, 138-146.
10. Piozzi, F.; Passannanti, S.; Paternostro, M. P.; Sprio, V. *Phytochemistry* **1971**, *10*, 1164-1166.
11. Ferraro, G.; Martino, V.; Borrajo, G.; Coussio, J. D. *Phytochemistry* **1987**, *26*, 3092-3093.
12. Bohlmann, F.; Ziesche, J.; Robinson, H.; King, R. M. *Phytochemistry* **1981**, *20*, 267-270.
13. a) Romo de Vivar, A.; Guerrero, C.; Díaz, E.; Bratoeff, E. A.; Jiménez, L. *Phytochemistry* **1976**, *15*, 525-527. b) Romo de Vivar, A.; Delgado, G.; Guerrero, C.; Reséndiz, J.; Ortega, A. *Rev. Latinoamer. Quím*. **1978**, *9*, 171-176. c) Hoeneisen, M.; Silva, M.; Bohlmann, F. *Phytochemistry* **1980**, *19*, 2765-2766
14. a) Samek, Z.; Harmatha, J. *Coll. Czech. Chem. Commun*. **1978**, *43*, 2779-2799. b) Samek, Z. *Tetrahedron* **1970**, *9*, 671-674.
15. Kametani, T.; Yukawa, H.; Suzuki, Y.; Honda, T. *J. Chem. Soc., Perkin Trans. 1*, **1985**, 2151.
16. Kijoa, A.; Bastos, M. S. M.; Gedris, T.; Herz, W. *Phytochemistry* **1993**, *32*, 383-385.
17. a) Bohlmann, F.; Ziesche, J.; King, R. M.; Robinson, H. *Phytochemistry* **1981**, *20*, 263-266. b) Singh, P.; Sharma, A. K.; Joshi, K. C.; Jakupovic, J.; Bohlmann, F. *Phytochemistry* **1985**, *24*, 2023-2028.
18. a) Maragelman, K. M.; Anza Espinar, L.; Sosa, V. E.; Uriburu, M. L.; de la Fuente, J. R. *Phytochemistry* **1996**, *41*, 499-502. b) Da Costa, F. B.; Ito, Y.; André, R. F. G.; Vichnewski, W. *Fitoterapia* **1998**, *69*, 86-87. Delgado, G.; Álvarez, L.; Romo de Vivar, A. *Phytochemistry* **1985**, *24*, 2736-2738.
19. Bohlmann, F.; Ziesche, J.; Robinson, H.; King, R. *Phytochemistry* **1981**, *20*, 267-270.
20. Delgado, G.; Guzmán, S. *Synlett* **1999**, 1006-1008.
21. Delgado, G.; Tejeda, V.; Salas, A.; Chávez, M. I.; Guzmán, S.; Bolaños, A.; Aguilar, M. I.; Navarro, V.; Villarreal, M. I. *J. Nat. Prod*. **1998**, *61*, 1082-1085.
22. The transformation of a germacrolide to a melampolide has been also previously observed during the characterization of the aldehyde derived from deacetyl gochnatolide [23], which isomerized to 8-*epi*-schkuhriolide
23. Maldonado, E.; Flores, A. M.; Ortega, A. *Phytochemistry* **1988**, *27*, 861-863.
24. Delgado, G.; Álvarez, L.; Guzmán, S. *Trends in Organic Chemistry* (India) **1995**, *5*, 1-10
25. Fischer, N. H.; Olivier, N. J.; Fischer, H. D. *Progress in the Chemistry of Organic Natural Products.* Herz, W.; Griesebach, H.; Kirby, G. W. Eds. Springer-Verlag. Wien, New York. **1979**, *38*, 47-390.
26. Schmidt, T. J. *Curr. Org. Chem*. **1999**, *3*, 577-608.
27. a) Siedle, B.; García-Piñeres, A. J.; Murillo, R.; Schulte-Möntig, J.; Castro, V.; Rüngeler, P.; Klaas, Vh. A.; Da Costa, F.; Kisiel, W.; Merfort, I. *J. Med. Chem*. **2004**, *47*, 6042-6054. b) Humar, M.; García-Piñeres, A. J.; Castro, V.; Merfort, I. *Biochem. Pharmacol*. **2003**, *65*, 1551-1563.
28. a) Taylor, P. G.; Depuy Loo, O. A.; Bonilla, J. A.; Murillo, R. *Fitoterapia* **2008**, *79*, 428-432. b) Nicolete, R.; Arakawa, N. S.; Rius, C.; Nomizo, A.; Jose, P. J.; Da Costa, F.; Sanz, M. J.; Faccioli, L. H. *Phytomedicine* **2009**, *16*, 904-915.

29. a) Tubaro, A.; Dri, P.; Delbello, G.; Zilli, C.; Della Logia, R. *Agents Actions* **1985**, *17*, 347-349. b) Della Logia, R.; Tubaro, A.; Sosa, S.; Becker, H.; Saar, St.; Isaac, O. *Planta Med*. **1994**, *60*, 516-529. c) Delgado, G.; Olivares, M. S.; Chávez, M. I.; Ramírez-Apan, T.; Linares, E.; Bye, R. A.; Espinosa-García, F. J. *J. Nat. Prod*. **2001**, *64*, 861-864.

30. a) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paul, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. *J. Natl. Cancer Inst*. **1991**, *83*, 757-766. b) García, A.; Ramírez-Apan, T.; Cogordán, J. A.; Delgado, G. *Can. J. Chem*. **2006**, *84*, 1593-1602.