Natural Product Research: Formerly Natural Product Letters
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gnpl20

Synthesis and antimalarial activities of optically active labdane-type diterpenes

José Villamizar [a], Jean P. Pittelaud [b], Juan R. Rodrigues [c], Neira Gamboa [c], Nieves Canudas [b], Eleonora Tropper [a], Franklin Salazar [a] & Juan Fuentes [b]

[a] Centro de Química, Instituto Venezolano de Investigaciones Científicas (IVIC), Apartado 21827, Caracas 1020-A, Venezuela

[b] Departamento de Química, Universidad Simón Bolívar, Caracas, Venezuela

[c] Facultad de Farmacia, Universidad Central de Venezuela, Caracas, Venezuela

Published online: 29 Oct 2009.

To cite this article: José Villamizar , Jean P. Pittelaud , Juan R. Rodrigues , Neira Gamboa , Nieves Canudas , Eleonora Tropper , Franklin Salazar & Juan Fuentes (2009) Synthesis and antimalarial activities of optically active labdane-type diterpenes, Natural Product Research: Formerly Natural Product Letters, 23:10, 891-902, DOI: 10.1080/14786410802164048

To link to this article: http://dx.doi.org/10.1080/14786410802164048

Natural Product Research
Vol. 23, No. 10, 10 July 2009, 891–902

Taylor & Francis
Taylor & Francis Group

Synthesis and antimalarial activities of optically active labdane-type diterpenes

José Villamizar[a]*, Jean P. Pittelaud[b], Juan R. Rodrigues[c], Neira Gamboa[c], Nieves Canudas[b], Eleonora Tropper[a], Franklin Salazar[a] and Juan Fuentes[b]

[a]*Centro de Química, Instituto Venezolano de Investigaciones Científicas (IVIC), Apartado 21827, Caracas 1020-A, Venezuela;* [b]*Departamento de Química, Universidad Simón Bolívar, Caracas, Venezuela;* [c]*Facultad de Farmacia, Universidad Central de Venezuela, Caracas, Venezuela*

(*Received 20 February 2006; final version received 30 April 2009*)

An efficient method for the synthesis of optically active labdane-type diterpenes from (+)-manool **8** is described. We prepared the natural labdane-type diterpene **5** via key intermediate peroxide **9**, and synthetic hydroxybutenolides **6** and **7** via a furan photosensitised oxygenation reaction of labdafuran (**14**). Compounds **5**, **6**, **7** and **9** were evaluated as inhibitors of the β-haematin formation and globin proteolysis, and then were assayed in a malarial murine model. Compound **9** was the most promising compound, showing a positive correlation between *in vitro* and *in vivo* activities.

Keywords: labdane-type diterpenes; *Hedychium coronarium*; synthesis; antimalarial activities

1. Introduction

Malaria is distributed throughout the tropical and sub-tropical regions of the world and causes more than 300 million acute illnesses and at least 1 million deaths annually (WHO Report, 1999). Malaria parasites are developing unacceptable levels of resistance and this can be attributed to the use of a single drug for treatment and the adaptation of the malarial parasite by developing alternate pathways for survival. The present strategy for new drug development is directed towards identifying the essential enzyme systems in the parasite and developing molecules to inhibit them (Bodeker & Willcox, 1996; Ridley et al., 1996). The evaluation of medicinal plants used in the preparation of folk remedies has provided effective compounds with novel structures for the treatment of diseases caused by protozoan parasites. In fact, the most effective antimalarials have originated from plants: quinine from the bark of the Peruvian *Cinchona* tree, and artemisinin from the Chinese antipyretic *Artemisia annua* L. (Bodeker & Willcox, 1996; Ridley et al., 1996).

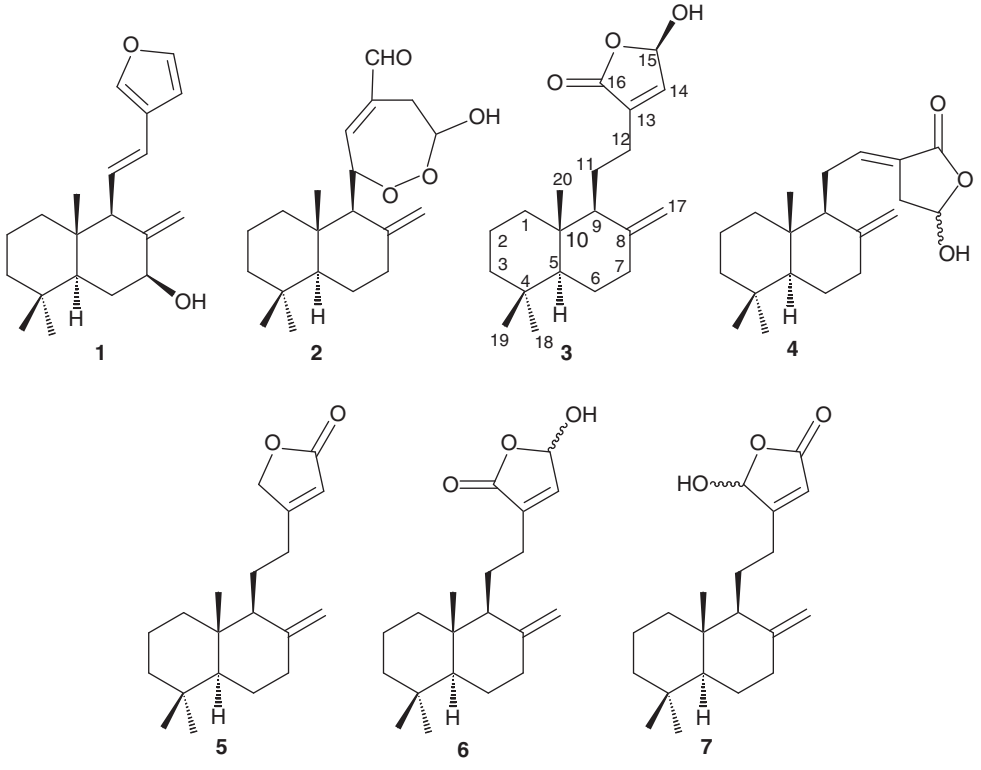
Labdane diterpenoids are among the most common types of diterpenes isolated from plants and sponges (Hanson, 1999, 2000). These classes of compounds are interesting for their cytotoxic, antifungal, anti-inflammatory, antiparasitic and analgesic properties (Dimas et al., 1998; Itokawa & Morita, 1988). However, as in the case of many other

*Corresponding author. Email: jvillami@ivic.ve

ISSN 1478–6419 print/ISSN 1029–2349 online

DOI: 10.1080/14786410802164048
http://www.informaworld.com

natural products, they can be isolated only in minute amounts, limiting the study of biological activities. *Hedychium coronarium* Koeng has been cultivated in Japan, China, India and Brazil. The rhizome of *H. coronarium* has been used for the treatment of headache, sharp pain and rheumatism (Dimas et al., 1998; Itokawa & Morita, 1988). Several labdane-type diterpenes have been isolated from this herbal medicine. In 1988, Itokawa et al. isolated the labdane-type diterpenes coronarin A (**1**), B (**2**), C (**3**) and D (**4**) from the rhizomes of *H. coronarium* cultivated in Brazil, showing cytotoxic activity against Chinese hamster V-79 cells. In 2002, Matsuda, Morikawa, Sakamoto, Toguchida and Yoshikawa isolated the labdane-type diterpene labda-8(17),13(14)-dien-15,16-olide (**5**) from the methanolic extract of a fresh rhizome of *H. coronarium* cultivated in Japan, showing anti-inflammatory activity (Morikawa et al., 2002). In 2003, Scio et al. isolated labdane-type diterpenes from *Alomia myriadenia*, showing cytotoxic and trypanocidal activity. Previously, Scio et al. (2002) have reported that some labdane-type diterpenes isolated from *A. myriadenia* showed strong activity against *Plasmodium falciparum*, one of the ethiological agents of human malaria.



To date, a number of semi-syntheses of these biologically active labdane-type diterpenoids have been reported by employing (−)-sclareol as a starting material (Jung, Ko, & Lee, 1998a; Jung et al., 1998b; Müller, Schröder, Magg, & Seifert, 1998). In 1982, Nakano, Martin and Rojas reported the synthesis of some labdane-type diterpenes, such as lactone **5** from (+)-manool **8**, but with poor yield. Recently, we have developed a new highly efficient synthesis of optically active labdane-related natural products. The key reaction consists of the dehydration of commercially available (+)-manool **8** and the

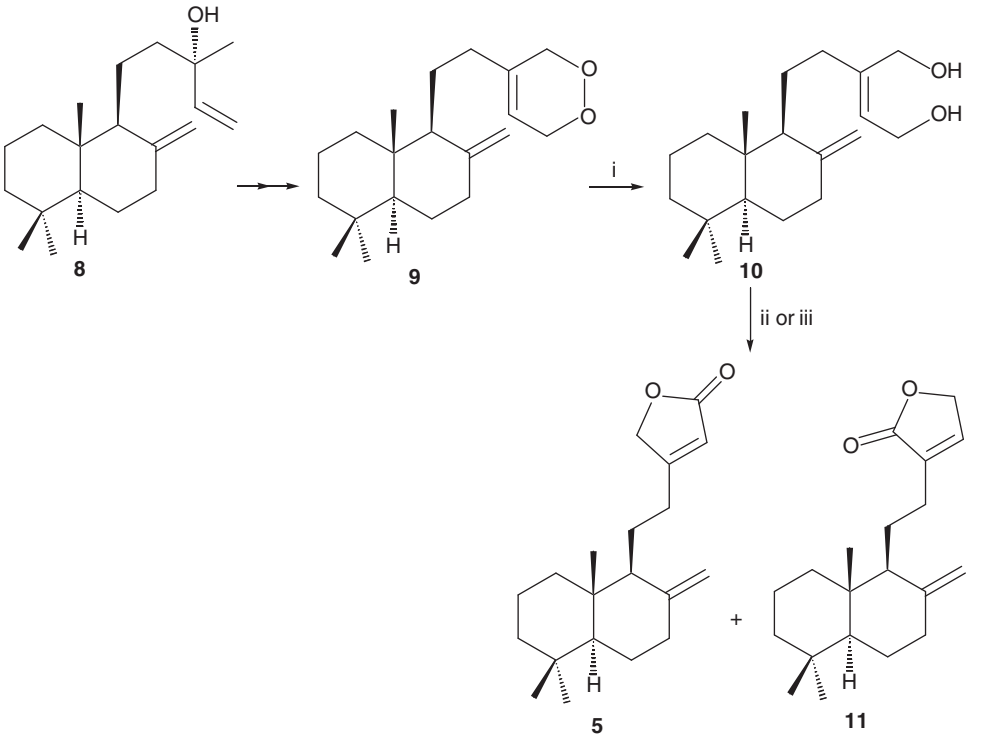
photooxidation reaction of the resulting diene to the peroxide **9** (Villamizar, Fuentes, Salazar, Tropper, & Alonso, 2003). Continuing with our research into the synthesis of the labdane-type diterpenes, we have been interested in the development of synthetic routes to the natural labdane-type diterpene **5** and synthetics **6** and **7**, and a screening search of new antimalarial agents with novel structures.

2. Results and discussion

2.1. *Synthesis of labdane-type diterpenes*

The first step of the synthetic sequence involves reduction of peroxide **9**, whose efficient synthesis from (+)-manool **8** has been previously reported by the present authors (Villamizar et al., 2003). Reduction of compound **9** with $LiAlH_4$ afforded the labda-8(17),13(*Z*)-diene-15,16-diol (**10**). In order to synthesise the title compound **5**, compound **10** was submitted to oxidative lactonisation. A satisfactory completion of the synthesis of lactone **5** required a selective oxidation of the C-15 hydroxymethyl group of the diol **10**. Oxidation with tetra-*n*-propylammonium perruthenate (TPAP) (Ley, Norman, Griffith, & Marsden, 1994) yielded a mixture of lactones **5** and **11**, as evidenced from the ^{1}H NMR spectrum. Separation of this mixture over silica gel failed. It has been found that silver carbonate absorbed on celite (Fétizon's reagent) (Fétizon, Golfier, & Louis, 1969) is a neutral oxidising agent which selectively transforms primary diols to lactones. Oxidation of compound **10** with Fétizon's reagent (Fétizon et al., 1969) afforded the desired lactone **5** in good yield, whose physical and spectroscopic properties were identical with those reported (Matsuda et al., 2002; Morikawa et al., 2002; Nakano et al., 1982), and only a small amount of isomeric lactone **11** (Scheme 1).

Compound **14** was prepared recently by the present authors by different methods (Villamizar et al., 2003). In an attempt to increase the yield of the compound **14**, we first prepared the alcohol **12** (Villamizar et al., 2003). Bromination of alcohol **12** with carbon tetrabromide and triphenylphosphine (Ph_3P) under neutral conditions gave the corresponding bromide **13**. The nucleophilic addition of the organolithium compound, derived from 3-bromofuran, to the bromide (**13**) afforded the desired compound **14**, whose physical and spectroscopic properties were identical with those reported (Villamazir et al., 2003). With compound **14** in hand, we continued the oxidation of furan ring to obtain the desired hydroxybutenolides. The photooxidation of 3-substituted furans is not regiospecific, yielding both the 2-alkyl-4-hydroxy- and the 3-alkyl-4-hydroxybutenolide regioisomers (Feringa, 1987; Kernan & Faulkner, 1988). Irradiation of labdafuran (**14**) in THF (external 150 W halogen–tungsten lamps, Pyrex well) in the presence of oxygen and a catalytic amount of Rose Bengal afforded a mixture of two regioisomeric hydroxybutenolides, **6** and **7**. Both lactones were separated by crystallisation from hexane in a 32 and 21% yield, respectively (Scheme 2). The ^{1}H NMR spectrum of **6** (Table 1) was consistent with a α-alkyl-substituted-15-hydroxybutenolide, similar to natural coronarin C **3** (Dimas et al., 1998; Itokawa & Morita, 1988). The presence of signals at 6.81 ppm, which must be assigned to proton H-14, placed at the β-position of an α,β-unsaturated butenolide, and the signal at 6.08 ppm assigned to H-15. The ^{1}H NMR spectrum of regioisomeric hydroxybutenolide derivative **7**, the most deshielded signal appeared at 5.95 ppm attributable to H-16, and the signal at 5.83 ppm assigned to H-14, therefore the structure of α,β-alkyl-substituided-16-hydroxidebutenolide has been assigned to this compound. The formation of compounds **6** and **7** was deduced by thermal decomposition



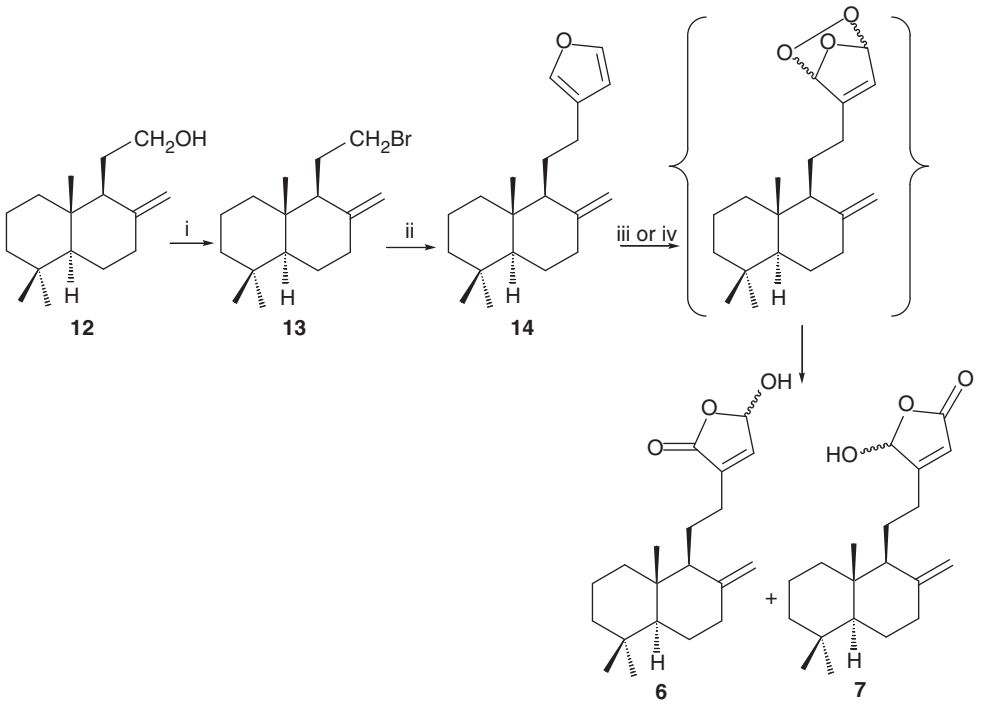
Scheme 1. Reagents and conditions: (i) $LiAlH_4$, THF, reflux (96%); (ii) TPAP, *N*-methylmorpholine *N*-oxide, CH_2Cl_2 (mixture of **5** and **11**); (iii) Ag_2CO_3-Celite, Benzene, r.t. (96% of **5**; 3% of **11**).

of the unstable endoperoxide, which result from [4 + 2] addition of singlet oxygen to labdafuran (**14**) (Feringa, 1987; Kernan & Faulkner, 1988). In an attempt to increase the yield of the photooxidation reaction, we irradiated labdafuran (**14**) in $CHCl_3$ (external 150 W halogen–tungsten lamp) in the presence of oxygen, 2,6-lutidine and a catalytic amount of *meso*-tetraphenylporphine, which afforded a mixture of two regioisomeric hydroxybutenolides **6** and **7** (Scheme 2). The one which formed in larger amounts (54%) was found to be hydroxybutenolide **7**, and the other (5%) was found to be **6**. The major formation of compound **7** was deduced by regiospecific removal of the hydrogen at C-15 on the intermediate endoperoxide, with a hindered base at low temperature in order to favour base-catalysed decomposition rather than thermal decomposition (Feringa, 1987; Kernan & Faulkner, 1988).

As none of the signals were doublets in the ^{1}H NMR spectrum of compounds **6** and **7**, while in the ^{13}C NMR spectrum no additional signals were observed, only one of the two possible C-15 or C-16 epimers in compounds **6** and **7** respectively was present. However, the configuration at C-15 or C-16 could not be determined.

2.2. *Biological activity*

Plasmodium parasite proteases degrade the host haemoglobin in order to get the aminoacids for its protein synthesis (Goldberg, 1992). The free haem moieties produced



Scheme 2. Reagents and conditions: (i) CBr_4, Ph_3P, THF, r.t.; (ii) 3-bromofuran, *n*-BuLi, THF, −78°C; (iii) O_2, *hv*, Rose Bengal, THF, −0°C, 2 h (32% of **6**; 21% of **7**); (iv) O_2, *hv*, *meso*-tetraphenylporphine, 2,6-lutidine, $CHCl_3$, −78°C, 2 h (5% of **6**; 54% of **7**).

Table 1. Biological results of the labdane-type diterpenes assayed.

Compound	% IHF	% IGP	% P
5	77.68 ± 2.93^a	44.85 ± 2.97	24.2 ± 4.85
6	<5	65.01 ± 1.33	19.06 ± 1.67
7	<5	95.6 ± 0.42^b	18.89 ± 2.23
9	50.81 ± 3.61	23.89 ± 1.85	4.4 ± 1.91^c
Saline	NA	NA	20 ± 2.86
CQ	76.82 ± 3.92	26.92 ± 1.85	3.66 ± 0.66
PEP	NA	93.77 ± 0.67	NA
LEP	NA	90.0 ± 0.69	NA

Notes: CQ: chloroquine; LEP: leupeptin (cysteine protease inhibitor); PEP: pepstatin (aspartic protease inhibitor); IHF: inhibition of β-haematin formation; IGP: inhibition of globin proteolysis; P: parasitaemia. The results are expressed by the mean ± standard error of the mean. [a]$p > 0.05$ compared to CQ. [b]$p < 0.0001$ and $p < 0.05$ compared to LEP and PEP, respectively. [c]$p < 0.01$ compared to saline and $p > 0.05$ compared to CQ-treated mice. NA: not applicable.

spontaneously crystallise in the acid environment of the digestive vacuole. This mechanism guarantees the parasites' survival due to the fact that free haem is highly toxic to these parasites because of its oxidant properties (Fitch et al., 1982; Orijh & Fitch, 1993). Compounds which inhibit the β-haematin formation and the haemoglobin degradation might be potential antimalarials.

To evaluate the biological activities of the compounds, we test **5**, **6**, **7** and **9** to inhibit the haem polymerisation and the globin proteolysis and we evaluated the effects on a malarial murine model (Table 1).

Compound **5** inhibited the β-haematin formation without significant differences compared to chloroquine control ($p > 0.05$), while **9** showed a moderate inhibition. This result may suggest the relevance of a lactone group to interfere with this process. In addition, all of the compounds tested were active as inhibitors of the globin proteolysis. Compound **7** was the most effective: even better than leupeptin ($p < 0.0001$) and pepstatin ($p < 0.05$). On the other hand, the importance of the regioisomery arrangement might be suggested by the lowered activity showed by **6**.

Particular attention must be paid to **9**, which was able to decrease the parasitemia levels as well as chloroquine did ($p > 0.05$). We can assume that this antimalarial activity obeys a different mechanism of actions, as our results showed moderate inhibition of β-haematin formation and globin proteolysis; in fact, it is noticeable that this compound is an endoperoxide-labdane and it is well known that endoperoxide-related structures, such as artemisinin and its derivatives, are potent antimalarials (Meshnick, Taylor, & Kamchonwongpaisan, 1996; Olliaro, Haynes, Meunier, & Yuthavong, 2001).

In summary, we have synthesised and identified new active labdane-type diterpenes as antimalarial compounds, which act by inhibiting the β-haematin formation and/or the globin proteolysis. Some of them showed a significant inhibition of one process or a moderate inhibition of both. Compound **9** was the most interesting compound, showing a positive correlation between *in vitro* and *in vivo* activities.

3. Experimental

3.1. *General*

Melting points were measured with a Kofler hot-stage apparatus and were uncorrected. NMR spectra were recorded with Bruker Avance-300 and Avance-500 spectrometers. IR spectra were recorded using a Nicolet Magna 560 FT-IR spectrometer. High-resolution mass spectra (HRMS) were obtained on a JEOL JMS-AX505WA mass spectrometer. The intensity of each peak in the mass spectrum relative to the base peak is reported in parentheses. Optical rotations were obtained for $CHCl_3$ solutions on a Perkin-Elmer 341 polarimeter, and their concentrations are expressed in g/100 mL. Manool resin was purchased from Westchem Industries, Ltd. and purified to obtain (+)-Manool, $[\alpha]_D^{24} + 28$ (*c* 1.5, $CHCl_3$). THF, ether, DME and benzene were freshly distilled from Na-benzophenone before use. All other solvents and reagents were obtained from commercial suppliers and used without further purification. Merck silica gel (70–230 mesh ASTM) was used for column chromatography. TLC was performed on Analtech silica gel 60 G_{254} and the spots were observed either by exposure to iodine or by UV light. All organic extracts were dried over Na_2SO_4 and evaporated under reduced pressure below 60°C.

3.2. *Labda-8(17),13(Z)-diene-15,16-diol (10)*

To a suspension of $LiAlH_4$ (37.43 mg, 0.98 mmol) in dry THF (5 mL), peroxide **9** (0.250 g, 0.82 mmol) in THF (4 mL) was added dropwise at 0°C. This mixture was refluxed for 2 h, then water was added and extracted with ether. The solvent was evaporated under pressure

and the product was chromatographed over silica gel and when eluted with 50% ether in hexane, which afforded diol **3** (0.241 g, 96%) as white crystals (hexane): m.p. 119–120°C; $[\alpha]_D^{24} = +40^\circ$ ($c = 1.0$, $CHCl_3$), (Matsuda et al., 2002; Morikawa et al., 2002) $[\alpha]_D = 42^\circ$ ($c = 0.39$, $CHCl_3$); ν_{max}/cm^{-1}(KBr) 3602, 3079, 1642 and 900; δ_H (300 MHz; $CDCl_3$) δ 0.65, 0.77, 0.84 (3H each, s, CH_3), 4.15 (1H, d, $J = 12.9$ Hz, H-16), 4.17 (1H, d, $J = 12.9$ Hz, H-16), 4.18 (2H, bd, H-15), 4.49 (1H, bs, H-17), 4.80 (1H, bt, H-17) and 5.57 (1H, bt, $J = 6.9$ Hz, H-14); δ_C (75.45 MHz; $CDCl_3$) 14.47 (C-20), 19.34 (C-11), 21.69 (C-19), 22.09 (C-2), 24.41 (C-6), 33.58 (C-4), 33.58 (C-18), 34.63 (C-12), 38.31 (C-7), 39.06 (C-1), 39.67 (C-10), 42.11 (C-3), 55.48 (C-5), 56.39 (C-9), 58.50 (C-16), 60.80 (C-15), 106.29 (C-17), 126.05 (C-14), 144.47 (C-13) and 148.56 (C-8); *m/z* (EI) 306.2558 ($C_{20}H_{34}O_2$ requires 306.2560), 288 (20), 205 (75), 177 (65), 137 (100), 98 (86).

3.3. *Labda-8(17),13(14)-dien-15,16-olide (5)*

Method A: Diol **3** (0.110 g, 0.36 mmol) was dissolved in dichloromethane (3 mL) containing both 4 Å molecular sieves (0.200 g) and *N*-methylmorpholine *N*-oxide (63.27 mg, 0.54 mmol). After stirring the mixture for 10 min., tetra-*n*-propylammonium perruthenate (6.3 mg, 0.018 mmol) was added and the reaction was followed by TLC until complete. After the usual work-up, the crude product (0.105 g) was obtained. The NMR spectrum indicated that it consisted of a mixture of lactones **5** and **11**. Further purification over silica gel failed.

Method B: To a suspension of silver carbonate-celite (37.43 mg, 0.98 mmol) in dry benzene (3 mL) was added diol **10** (0.100 g, 0.32 mmol) in benzene (2 mL) at room temperature. The reaction mixture was filtered through silica gel and the filtrate was evaporated. The resulting crude product was chromatographed over silica gel and eluted with 50% ether in hexane, which afforded lactone **5** (95 mg, 96%), which after recrystallisation from hexane showed: m.p. 76–78°C; $[\alpha]_D^{24} = 39^\circ$ ($c = 1.4$, $CHCl_3$), (Nakano et al., 1982)] $[\alpha]_D = +41^\circ$ ($c = 1.2$, $CHCl_3$); ν_{max}/cm^{-1}(KBr) 1775, 1740, 1640 and 1630; δ_H (300 MHz; $CDCl_3$) 0.67, 0.78, 0.85 (3H each, s, CH_3), 4.42 (1H, br s, H-17), 4.66 (1H, dd, $J = 17.3$ and 1.7 Hz, H-16), 4.72 (1H, dd, $J = 17.3$ and 1.7 Hz, H-16), 4.84 (1H, br s, H-17), 5.81 (1H, t, $J = 1.6$ Hz, H-14); δ_C (75.45 MHz; $CDCl_3$) 14.36, 19.24, 21.20, 21.63, 24.32, 27.45, 33.51, 33.51, 38.14, 39.09, 39.70, 41.93, 55.42, 56.06, 73.08, 106.37, 115.07, 147.84, 171.06 and 174.17; *m/z* (EI) 302.2258 ($C_{20}H_{30}O_2$ requires 302.2255), 287 (15), 206 (34), 137 (71), 109 (100), 98 (92).

3.4. *5-(2-Bromo-ethyl)-1,1,4a-trimethyl-6-methylene-decahydro-naphthalene (13)*

To a solution of alcohol **12** (0.2 g, 0.84 mmol) in THF (6 mL) was added PPh_3 (0.88 g, 3.36 mmol) and CBr_4 (0.83 g, 2.50 mmol), and the whole mixture was stirred for 20 min at room temperature. The reaction mixture was diluted with brine and extracted with ether. The solvent was evaporated under reduced pressure and the product was chromatographed over silica gel and eluted with hexane, which afforded bromide **13** (0.21 g, 83%) as colourless oil; δ_H (300 MHz; $CDCl_3$) 0.67, 0.78, 0.86 (3H each, s, CH_3), 2.38 (1H, ddd, $J = 13$, 4, 2 Hz), 3.25 (1H, ddd, $J = 8.6$, 8, 7 Hz, H-12), 3.51 (1H, ddd, $J = 8.6$, 8, 4 Hz, H-12), 4.45 (1H, br s, H-17), 4.82 (1H, br s, H-17); δ_C (75.45 MHz; $CDCl_3$) 14.81, 19.4, 21.8, 24.42, 28.06, 33.61, 33.61, 33.75, 38.22, 39.10, 39.64, 42.11, 55.33, 55.42, 106.20 and 147.71; *m/z* (EI) 298.1299 ($C_{16}H_{27}Br$ requires 298.1296).

3.5. *15,16-Epoxy-8(17),13(16),14-labdadiene (14)*

To a cooled solution of the 3-bromofuran (0.163 g, 1.1 mmol) in dry THF (3 mL) at −78°C was added *n*-butyllithium (0.6 mL, 1.6 M in hexane). The resulting brown solution was stirred for 10 min at −78°C and then a solution of bromide **13** (0.121 g, 0.51 mmol) in THF (2 mL) was added dropwise. After this mixture had been stirred for 2 h at −78°C, excess H_2O was added at room temperature with additional stirring for 30 min. The product was extracted with ether, dried, and the solvent was evaporated under reduced pressure and the product was chromatographed over silica gel and eluted with 5% diethyl ether in hexane, which afforded furanolabdane **14** (0.192 g, 93%) as colourless oil; $[\alpha]_D^{24} = +23°$ ($c = 2.0$, $CHCl_3$), (Dimas et al., 1998; Itokawa et al., 1988) $[\alpha]_D = 22°$ ($c = 0.14$, $CHCl_3$); ν_{max}/cm^{-1}(KBr) 3050, 1635, 1495, 870 cm^{-1}; δ_H (300 MHz; $CDCl_3$) 0.67, 0.78, 0.85 (3H each, s, CH_3), 2.23 (1H, m, H-12), 2.39 (1H, m, H-7), 2.54 (1H, m, H-17), 4.55 (1H, bs, H-17), 4.84 (1H, bs, H-17), 6.25 (1H, bs, H-14), 7.18 (1H, bs, H-16) and 7.33 (1H, t, H-15); δ_C (75.45 MHz; $CDCl_3$) 14.47 (C-20), 19.36 (C-11), 21.70 (C-19), 23.59 (C-12), 24.05 (C-2), 24.42 (C-6), 33.55 (C-4), 33.55 (C-18), 38.30 (C-7), 38.98 (C-1), 39.56 (C- 10), 42.10 (C-3), 55.42 (C-5), 56.05 (C-9), 106.23 (C-17), 110.94 (C-14), 125.59 (C-13), 138.62 (C-16), 142.58 (C-15) and 148.51 (C-8); GC/MS *m*/*z* 286 (M^+, 31), 271 (9), 253 (3), 191 (27), 135 (26), 95 (100), 67 (18) and 41 (13); *m*/*z* (EI) 286.2290 ($C_{20}H_{30}O$ requires 286.2299), 271 (9), 191 (27), 137 (80), 95 (100), 67 (18).

3.6. *Labda-8(17),13-diene-15-hydroxy-15,16-olide 6 and Labda-8(17),13-diene-16-hydroxy-15,16-olide (7)*

Method A: A solution of labdafuran (**14**) (0.105 g; 0,37 mmol) in THF (10 mL), containing Rose Bengal (1 mg), was irradiated at 0°C with an external 150 W halogen–tungsten lamp for 2 h, during which time oxygen was bubbled through the reaction mixture. The solvent was evaporated under reduced pressure and the residue chromatographed over silica gel and eluted with 15% ethyl acetate in hexane, which afforded a mixture of compounds **6** and **7**, as a colourless oil (as evidenced by the NMR spectrum). Crystallisation from hexane gave pure compound **7** (25 mg; 21%): m.p. 89–91°C; $[\alpha]_D^{24} = +48°$ ($c = 0.7$; $CHCl_3$). ν_{max}/cm^{-1}(KBr) 3364, 3015, 1645, 1762; δ_H (300 MHz; $CDCl_3$) 0.67 (3H, s, CH_3); 0.78 (3H, s, CH_3); 0.85 (3H, s, CH_3); 1.31 (1H, m, H-6); 1.72 (1H, m, H-6); 1.93 (1H, m, H-7); 2.37 (1H, m, H-7); 4,45 (1H, bs, H-17); 4.84 (1H, bs, H-17); 5.83 (1H, s, H-14); 5.95 (1H, bs, H-16); δ_C (75.45 MHz; $CDCl_3$) 14.41 (C-20); 19.31 (C-11); 20.82 (C-2); 21.69 (C-19); 24.39 (C-6); 26.76 (C-12); 33.57 (C-4); 33.57 (C-18); 38.20 (C-7); 39.14 (C-10); 39.78 (C-1); 42.04 (C-3); 55.49 (C-5); 56.33 (C-9); 99.02 (C-16); 106.51 (C-17); 117.14 (C-14); 147.91 (C-8); 170.41 (C-13); 171.57 (C-15); *m*/*z* (EI) 318.1970 ($C_{20}H_{30}O_3$ requires 318.2193) (23), 300 (15), 204 (19), 177 (25), 137 (100), 95 (37).

Chromatography of the mother-liquid of compound **6** over silica gel with 15% ethlyl acetate in hexane afforded pure compound **6** (37.6 mg, 32%) as an oil; $[\alpha]_D^{24} = +30°$ ($c = 0.6$; $CHCl_3$); ν_{max}/cm^{-1}(KBr) 3426, 3015, 1643, 1763; δ_H (300 MHz; $CDCl_3$) 0.65 (3H, s, CH_3); 0.77 (3H, s, CH_3); 0.84 (3H, s, CH_3); 4.51 (1H, bs, H-17); 4.83 (1H, bs, H-17); 6.08 (1H, bs, H-15); 6.81 (1H, d, $J = 1.5$ Hz, H-14); δ_C (75.45 MHz; $CDCl_3$) 14.41 (C-20); 19.32 (C-2); 21.47 (C-12); 21.69 (C-19); 24.38 (C-6); 24.48 (C-11); 33.56 (C-4); 33.56 (C-18); 38.21 (C-7); 39.11 (C-1); 39.70 (C-10); 42.08 (C-3); 55.49 (C-5); 56.45 (C-9); 97.08 (C-15); 106.56 (C-17); 139.02 (C-13); 142.85 (C-14); 147.97 (C-8); *m*/*z* (EI) 318.2284 ($C_{20}H_{30}O_3$ requires 318.2193) (20), 303 (21), 177 (23), 137 (100), 95 (37).

Method B: A solution of labdafuran (**14**) (0.1 g; 0.35 mmol) in CH_2Cl_2 (10 mL), containing *meso*-tetraphenylporphine (1 mg) and diisopropylamine (10 eq), was irradiated at −78°C with an external 150 W halogen–tungsten lamp for 2 h, during which time oxygen was bubbled through the reaction mixture. The solution was warmed to 23°C, and saturated aqueous oxalic acid (3 mL) was added. After 30 min of vigorous stirring, water (15 mL) and CH_2Cl_2–methanol (3 : 1, 50 mL) were added to the colourless mixture, and the aqueous portion was extracted with CH_2Cl_2–methanol (3 : 1, 2 × 50 mL). The solvent was evaporated under reduced pressure and the residue was chromatographed over silica gel. Crystallisation from hexane gave pure compound **7** (60.1 mg, 54%) as white crystals. Chromatography of the mother-liquid of compound **6** over silica gel with 15% ethlyl acetate in hexane afforded the pure compound **6** (5 mg, 5%).

3.7. *Determination of the antimalarial activity*

3.7.1. *Inhibition of the* β-*haematin formation*

The inhibition of the *β*-haematin formation was followed according to a slightly modified method of Baelmans, Deharo, Muñoz, Sauvain and Gisnburg (2000). Briefly, a solution of hemin chloride (50 μL; 5.2 mg mL^{-1}) was distributed in 96-well micro plates. Different concentrations of the compounds dissolved in DMSO (5–100 mM), were added in triplicate in test wells (50 μL) to a final concentration of 25–0.25 mM. Controls contained either water (50 μL), DMSO (50 μL) or chloroquine (50 μL, 2.5 mM). *β*-Haematin formation was initiated by the addition of acetate buffer (100 μL 0.2 M, pH 4.4). Plates were incubated (37°C × 48 h) to allow completion of the reaction and centrifuged (4000 RPM × 15 min, IEC-CENTRA, MP4R). After discarding the supernatant, the pellet was washed twice with DMSO (200 μL) and finally dissolved in NaOH (200 μL, 0.2 N). The solubilised aggregates were further diluted 1 : 2 with NaOH (0.1 N) and absorbance recorded at 405 nm (Microplate Reader, BIORAD-550). The results were expressed as a percentage of inhibition of *β*-haematin formation.

3.7.2. *Experimental host and strain maintenance*

Male albino mice (National Institute of Hygiene strain), weighing 18–22g, were maintained on a commercial pellet diet and housed under conditions approved by the Ethics Committee of the Faculty of Pharmacy, Central University of Venezuela. *Plasmodium berghei* (ANKA strain), a rodent malarial parasite, was used for infection. Mice were infected by i.p. passage of 10^7 infected erythrocytes diluted in phosphate buffered saline solution (PBS 10 mM, pH 7.4, 0.1 mL). Parasitemia was monitored by microscopic examination of Giemsa-stained smears.

3.7.3. *Parasite extracts*

Blood of infected animals at high levels of parasitemia (30–50%) was collected by cardiac puncture with a heparinised syringe, and the blood was centrifuged (500 *g* × 10 min, 4°C). Plasma and buffy coat were removed, and the red blood cell (RBC) pellet was washed twice with chilled PBS–glucose (5.4%). The washed RBC pellet was centrifuged on a discontinuous percoll gradient (80–70% percoll in PBS–glucose, 20,000 *g* × 30 min, 4°C). The upper band (mature forms) was removed

by aspiration, collected in Eppendorf tubes and washed twice with chilled PBS–glucose, and the infected RBC were lysed with the non-ionic detergent saponin (0.1% in PBS × 10 min). Cold PBS (1 mL) was added, and the samples were centrifuged (13,000 *g* × 5 min, 4°C) to remove erythrocyte cytoplasmic content (including erythrocyte haemoglobin). The free parasites were mixed with PBS–glucose (5.4%) and subjected to three freeze–thaw cycles (−70°C/+37°C). The final homogenate was used in the haemoglobin proteolysis assay.

3.7.4. *Mice native haemoglobin*

Native haemoglobin from non-infected mice was obtained by treating one volume of pellet erythrocytes with two volumes of water. The resulting lysate was used as the substrate in the haemoglobin proteolysis assay.

3.7.5. *Inhibition of globin proteolysis*

To assay the proteolytic activity of a trofozoite-rich extract, we incubated in a 96-well tissue culture plate (Greiner Bio-One): mice native haemoglobin (10 μL), parasite extract (50 μL), GSH (10 μL, 10 μM), and acetate buffer (0.2 M, pH 5.4) to a final volume of 100 μL. The compounds (5 mM) were incorporated in the incubation mixture dissolved in DMSO. After incubation (at 37°C × 18 h) the reactions were stopped by the addition of reduced sample buffer (upper buffer 25%, SDS 2%, β-mercaptoethanol 5%, glicerol 20%, bromophenol blue 0.0025%). The degree of digestion was evaluated electrophoretically by 15% SDS-PAGE (Rosenthal, 1995) followed by densitometric comparison of globin bands. DMSO, leupeptin and pepstatin controls were electrophoresed at the same time.

3.7.6. *Four day suppressive test*

The compounds were also tested in a malaria murine model according to Peters and Robinson (1999). Briefly, NIH male albino mice (18–23 g) were infected i.p. with 10^7 red blood cells infected with *P. berghei* ($n = 6$). Compounds were dissolved in DMSO (0.1 M), diluted with Saline–Tween 20 solutions (2%). Two hours after infection, each compound was administered once i.p. (20 mg kg^{-1}) for four consecutive days. On day 4, the parasitemia was counted by examination of Giemsa-stained smears. Chloroquine (25 mg kg^{-1}) was used as a positive control. The results were expressed as the percentage of parasitemia at the fourth day post-infection and were compared to the values reported by non-treated mice.

Data were statistically analysed using unpaired *t*-tests for specific group comparisons; assuming 95% confidence according GraphPad Prism 3.02.

Acknowledgements

The authors thank to CDCH (PG-CDCH-06.30.5125-2003) and Ms Diana Lourenco for support in the antimalarial evaluation. Decanato de Estudios Profesionales and Decanato de Investigación y Desarrollo of Universidad Simón Bolívar for the financial support. We thank Professor Julio Herrera (Universidad Simón Bolívar) for the measurement of mass spectra.

References

Baelmans, R., Deharo, E., Muñoz, E., Sauvain, M., & Gisnburg, H. (2000). Experimental conditions for testing the inhibitory activity of chloroquine on the formation of β-hematin. *Experimental Parasitology, 96*, 243–248.

Bodeker, G., & Willcox, M. (2000). New research initiative on plant-based antimalarials. *Lancet, 355*, 761.

Dimas, K., Demetzos, C., Marsellos, M., Sotiriadou, R., Malamas, M., & Kokkinopoulos, D. (1998). Cytotoxic activity of labdane type diterpenes against human leukemic cell lines *in vitro*. *Planta Medica, 64*, 208–211.

Feringa, B.L. (1987). Photooxidation of furans. *Recueil des Travaux Chimiques des Pays-Bas, 106*, 469–488.

Fétizon, M., Golfier, M., & Louis, J.-M. (1969). Oxidations by silver carbonate on celite XIII. Preparations of lactones. *Tetrahedron, 31*, 171–176.

Fitch, C.D., Chevli, R., Banyal, H.S., Phillips, G., Pfaller, M.A., & Krogstad, D.J. (1982). Lysis of *Plasmodium falciparum* by ferriprotoporphyrin IX and a chloroquine-ferriprotoporphyrin IX complex. *Antimicrobial Agents and Chemotherapy, 21*, 819–822.

Goldberg, D.E. (1992). Plasmodial hemoglobin degradation: An ordered pathway in a specialized organelle. *Infectious Agents and Disease, 1*, 207–211.

Hanson, J.R. (1999). Diterpenoids. *Natural Product Reports, 16*, 209–219.

Hanson, J.R. (2000). Diterpenoids. *Natural Product Reports, 17*, 165–174.

Itokawa, H., & Morita, H. (1988). Cytotoxic and antifungal diterpenes from the seeds of *Alpinia galanga*. *Planta Medica, 54*, 117–120.

Itokawa, H., Morita, H., Katou, T., Takeya, K., Cavalheiro, A.J., de Oliveira, R.C.B., et al. (1988). Cytotoxic diterpenes from the rhizomes of *Hedychium coronarium*. *Planta Medica, 54*, 311–315.

Jung, M., Ko, I., & Lee, S. (1998a). A concise conversion of (-)-sclareol into (+)-coronarin E and (-)-7-epi-coronarin A. *Journal of Natural Products, 61*, 1394–1396.

Jung, M., Ko, I., Lee, S., Choi, S.J., Youn, B.H., & Kim, S.K. (1998b). A concise synthesis and *in vitro* cytotoxicity of new labdane diterpenes. *Bioorganic & Medicinal Chemistry Letters, 8*, 3295–3298.

Kernan, M.R., & Faulkner, D.J. (1988). Regioselective oxidation of 3-alkylfurans to 3-alkyl-4-hydroxybutenolides. *Journal of Organic Chemistry, 53*, 2773–2776.

Ley, S.V., Norman, J., Griffith, W., & Marsden, S. (1994). Tetrapropylammonium perruthenate, $Pr_4N^+RuO_4^-$, TPAP: A catalytic oxidant for organic synthesis. *Synthesis, 7*, 639–665.

Matsuda, H., Morikawa, T., Sakamoto, Y., Toguchida, I., & Yoshikawa, M. (2002). Antiinflammatory principles and three new labdane-type diterpenes, hedychilactones A, B, and C, from the rhizome of *Hedychium coronarium* Koeng. *Heterocycles, 56*, 45–50.

Meshnick, S.R., Taylor, T.E., & Kamchonwongpaisan, S. (1996). Artemisinin and the antimalarial endoperoxides: From herbal remedy to targeted chemotherapy. *Microbiological Review, 60*, 301–315.

Morikawa, T., Matsuda, H., Sakamoto, Y., Ueda, K., Toguchida, I., & Yoshikawa, M. (2002). New farnesane-type sesquiterpenes, hedychiols A and B 8,9-diacetate, and inhibitors of degranulation in RBL-2H3 cells from the rhizome of *Hedychium coronarium*. *Chemical and Pharmaceutical Bulletin, 50*, 1045–1049.

Müller, M., Schröder, J., Magg, Ch., & Seifert, K. (1998). Synthesis of (+)-coronarin E. *Tetrahedron Letters, 39*, 4655–4656.

Nakano, T., Martin, A., & Rojas, A. (1982). Synthesis of labda-8(17),13(14)-dien-15,16-olide and 15,16-epoxylabda-8(17),13(16),14-triene and their rearrangements to clerodane derivatives. *Tetrahedron, 38*, 1217–1219.

Olliaro, P., Haynes, R., Meunier, B., & Yuthavong, Y (2001). Possible modes of action of the artemisinin-type compounds. *Trends in Parasitology, 14*, 122–126.

Orijh, A.U., & Fitch, C.S. (1993). Hemozoin production by *Plasmodium falciparum*: Variation with strain and exposure to chloroquine. *Biochimica et Biophysica Acta, 1157*, 270–274.

Peters, W., & Robinson, B.L. (1999). Chapter 92 – Malaria. In O. Zack & M. Sande (Eds.), *Handbook of animal models of infection* (pp. 757–773). London: Academic Press.

Ridley, R.G., Hofheinz, W., Matile, H., Jaquet, C., Dorn, A., Masciadri, S., et al. (1996). 4-aminoquinoline analogs of chloroquine with shortened side chains retain activity against chloroquine-resistant *Plasmodium falciparum*. *Antimicrobial Agents and Chemotherapy, 40*, 1846–1854.

Rosenthal, P.J. (1995). *Plasmodium falciparum*: Effects of proteinase inhibitors on globin hydrolysis by cultured malaria parasites. *Experimental Parasitology, 80*, 272–281.

Scio, E., Alves, T.M.A., Kretli, A.U., Soares, L., Shin, G.Y., Cordell, G.A., et al. (2002). Antiplasmodial and antitumoral activity of labdanes from *Alomia miryadenia* (Asteraceae). In 22nd IUPAC Symposium on the Chemistry of Natural Products, Sao Carlos, CP, Brazil, 4–8 September.

Scio, E., Ribeiro, A., Alves, T.M.A., Romahna, A.J., de S. Filho, J.D., Cordell, G., et al. (2003). Diterpenes from *Alomia myriadenia* (Asteraceae) with cytotoxic and trypanocidal activity. *Phytochemistry, 64*, 1125–1131.

Villamizar, J., Fuentes, J., Salazar, F.E., Tropper, E., & Alonso, R. (2003). Facile access to optically active labdane-type diterpenes from (+)-manool. Synthesis of (+)-coronarin E, (+)-15,16-epoxy-8(17),13(16),14-labdatriene, and (+)-labda-8(17),13(Z)-diene-15,16-diol. *Journal of Natural Products, 66*, 1623–1627.

WHO Report (1999). *Rolling back malaria* (pp. 49–63). World Health Organization: Geneva, Switzerland.