Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 2167–2169

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Antimalarial Compounds from *Parinari capensis*

Arina C. U. Uys,[a] Sarel F. Malan,[a,*] Sandra van Dyk[a] and Robyn L. van Zyl[b]

[a]*Pharmaceutical Chemistry, School of Pharmacy, Potchefstroom University for Christian Higher Education, Private Bag X6001, Potchefstroom 2520, South Africa*

[b]*Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown 2193, South Africa*

Received 14 February 2002; accepted 29 April 2002

Abstract—The antimalarial activity of the raw petroleum ether and dichloromethane extracts of the stems of *Parinari capensis* (Chrysobalanceae) was determined. Phytochemical investigation of these extracts led to the isolation of three diterpene lactones that possess antimalarial activity with IC_{50} values of 0.54, 0.67, and 1.57 μg/mL. Although their antimalarial activity is promising, the toxicity profiles of these diterpene lactones prevent further biological evaluation. They could however be used effectively as lead compounds in the synthesis of novel antimalarial agents.

Annually an estimated 300 million cases of malaria occur throughout the world, with 90% in sub-Saharan Africa. Mortality associated with the disease is estimated at over one million per year.[1]

Both *Parinari curatellifolia* and *Parinari excelsa* are used traditionally in the treatment of malaria and the antimalarial activity of the petroleum ether and dichloromethane extracts of *P. excelsa* was previously reported.[2–5] Although antifungal and antitumour activity were previously reported for the dichloromethane extract of the whole plant and the ethyl acetate extract of the root bark of *Parinari capensis*,[6,7] respectively, the antimalarial activity of this plant has, to the best of our knowledge, not yet been investigated. It was thus decided to investigate the antimalarial activity of *P. capensis* and to isolate and characterise the active compounds.

The antimalarial activity of the different extracts of *P. capensis* varied considerably (Table 1). Regardless of the solvent used, the stem extracts were more active than the extracts from the leaves. Since the petroleum ether and dichloromethane extracts of the stems were the most active, these extracts were selected for further study.

Three diterpene lactones were isolated from both the petroleum ether and the dichloromethane extracts (Fig. 1). Compound **1** was identified as 10-hydroxy-13-methoxy-9-methyl-15-oxo-20-norkaur-16-en-18-oic acid γ-lactone, compound **2** as 10,13-dihydroxy-9-methyl-15-oxo-20-norkaur-16-en-18-oic acid γ-lactone and compound **3** as 10-hydroxy-9-methyl-15-oxo-20-norkaur-16-en-18-oic acid γ-lactone (Fig. 1).

Compounds **1**–**3**[13] showed promising antimalarial activity, with compound **2** being the most active with an IC_{50} value of 0.54 ± 0.05 μg/mL (Table 2). The moderate antitumour activity previously reported[7] for these compounds made it necessary to determine the toxicity of these compounds. Toxicity, as measured on Graham cells, was quite high, with compound **1** being the most toxic with an IC_{50} value of 1.13 ± 0.07 μg/mL (Table 2).

Whole plants of *P. capensis* were collected in the Potchefstroom area during April and May 2000. Plants were positively identified by Mr. Bert Ubinck and Dr. Sarel Cilliers of the Department of Botany of the Potchefstroom University for Christian Higher Education. Dry powdered stems and leaves were extracted consecutively with petroleum ether, dichloromethane, ethyl acetate and ethanol for a period of 48 h with each solvent using a soxhlet extractor.

The concentrated, air dried extracts were tested for antimalarial activity and the most active extracts were separated by chromatography into pure compounds, which were spectroscopically identified and tested for antimalarial activity.

*Corresponding author. Fax: +27-18-299-4243; e-mail: fchsfm@puknet.puk.ac.za

0960-894X/02/$ - see front matter
PII: S0960-894X(02)00350-5

Plant extracts and pure compounds were assessed for in vitro antimalarial activity against the chloroquine-resistant FCR-3 strain of *Plasmodium falciparum*. The parasites were continuously cultured in vitro in a medium consisting of 10.4 g/L RPMI-1640, 5.9 g/L HEPES (*N*-2-hydroxyethyl-piperazine-*N*′-2-ethane-sulfonic acid), 4.0 g/L glucose, 44 mg/L hypoxanthine and 50 mg/L gentamycin with 10% (v/v) plasma and 0.21% (w/v) $NaHCO_3$.[8] Cultures were synchronised with 5% D-sorbitol when the parasites were in the ring stage as described by Lambros and Vandenberg.[9]

The inhibition of the in vitro uptake of [^{3}H]-hypoxanthine by malaria parasites was used as an indicator of parasite growth.[10] Extracts and isolated compounds were dissolved in DMSO and one in 10 dilutions were prepared and plated out in triplicate. The standard antimalarials, chloroquine and quinine, dissolved in sterilised water, were tested as positive controls. The experiment was repeated at least three times for each substance. DMSO controls were run to confirm that a 1% DMSO solution exhibited no antimalarial activity.

Table 1. Antimalarial activity of extracts from *P. capensis*

Extract	Antimalarial activity IC_{50}, μg/mL[a]
Stems	
Petroleum ether	1.11 (±0.35)
Dichloromethane	2.14 (±0.09)
Ethyl acetate	53.17 (±2.93)
Ethanol	187.79 (±42.79)
Leaves	
Petroleum ether	151.52 (±37.14)
Dichloromethane	65.16 (±2.98)
Ethyl acetate	143.33 (±60.98)
Ethanol	302.34 (±18.89)

[a]Means of three experiments, standard deviations in parentheses.

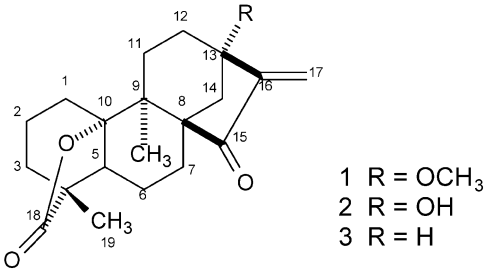

Figure 1. Diterpene lactone structure of compounds **1**–**3** isolated from the petroleum ether and dichloromethane extracts of the stems of *P. capensis*.

Table 2. Antimalarial activity and toxicity of isolated compounds **1**–**3**

	Antimalarial activity IC_{50}, μg/mL[a]	Toxicity IC_{50}, μg/mL[b]
1	0.67 (±0.18)	1.13 (±0.07)
2	0.54 (±0.05)	1.73 (±0.56)
3	1.57 (±0.41)	3.01 (±0.58)
Chloroquine	0.04 (±0.01)	nt
Quinine	0.17 (±0.02)	nt

[a]Means of three experiments.
[b]Means of two experiments, standard deviations in parentheses (nt, not toxic at 100 μg/mL).

The percentage parasitaemia and haematocrit were adjusted to 0.5 and 1.0%, respectively. Parasites were incubated for 24 h (at 37 °C in an anaerobic environment) before the addition of [^{3}H]-hypoxanthine and harvested 24 h later. After the second incubation period, the parasites were harvested onto glass fibre filter paper and the incorporated [^{3}H]-hypoxanthine was measured by liquid beta scintillation counting. The inhibitory effects of the substances were expressed as a percentage parasite growth of the untreated parasitised and erythrocyte controls.[10] These percentages were plotted against their respective concentrations and sigmoidal dose response curves were obtained after logarithmic transformation of the concentration. From the non-linear regression analysis the IC_{50} value (i.e., the concentration of agent corresponding to 50% parasite growth) was determined.

The transformation of the tetrazolium salt, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] to an insoluble formazan product was used to determine the toxicity of the isolated compounds.[11]

Human kidney epithelial (Graham cells) were cultured in Ham F10 medium containing 5% (v/v) heat inactivated fetal calf serum and 0.1% gentamycin. Cell density was adjusted to 0.5 million cells per millilitre, ensuring that at least 95% of the cells were viable before using them in the assay. Compounds were dissolved in DMSO to obtain stock solutions of ca. 10 mg/mL. One in 10 dilutions and appropriate DMSO controls were prepared and plated out in quadruplicate. After incubating test plates under humidified conditions at 37 °C, 5% CO_2 for 44 h MTT was added and the plates incubated for a further 4 h to ensure MTT cleavage. The reaction was stopped and the formazan crystals solubilised by the addition of DMSO.[12] Plates were shaken at 1020 rpm for 4 min before the absorbance was measured at a test wavelength of 540 nm and a reference wavelength of 690 nm. The experiment was duplicated. Results were expressed as a percentage cellular viability of the drug and cell-free controls.[11] Percentage cellular viability was plotted against concentration and sigmoidal curves were obtained after logarithmic transformation of the concentration. IC_{50} values were determined from these dose–response curves.

Separation of the crude petroleum ether and dichloromethane extracts of the stems of *P. capensis* was achieved with column- and thin-layer chromatography employing different mobile phases. Aliminium silica gel sheets (Alufolien 60 F_{254}, Merck) and silica gel (size 0.063–0.2 mm, Macherey Nagel) were used. TLC plates were inspected under UV light at a wavelenth of 366 nm.

Compound **1** was obtained after initial separation with a mobile phase of petroleum ether/dichloromethane/ethyl acetate/ethanol 10:2:2:1. After combining the polar fractions, compound **2** was obtained with a mobile phase that consisted of dichloromethane/ethyl acetate 2:1. Compound **3** was obtained from several nonpolar fractions after separation with a mobile phase

that consisted of petroleum ether/dichloromethane/ethyl acetate 5:1:1. All compounds were further purified by recrystallisation from ethanol. Approximately 320 mg (0.11% w/w) of compound **1**, 120 mg (0.04% w/w) of compound **2** and 55 mg (0.02% w/w) of compound **3** were obtained from 290 g ground stem material. Structure elucidation was done using NMR, MS and IR. Spectroscopic data and melting points of the isolated compounds correlated with the literature data.[6,7,13]

The three diterpene lactones isolated from the non-polar stem extracts of *P. capensis* showed promising antimalarial activity, with the IC_{50} value of compound **2** being comparable to that of quinine. Although these compounds are too toxic for in vivo or human use, they are substantially different from the quinolones in structure, and possibly their mode of action, and may thus be potentially useful as lead compounds for a new class of antimalarial drugs.

Acknowledgement

Mrs. Charlene van Zyl is thanked for her technical assistance with the Graham cells and the MTT assay.

References and Notes

1. World Health Organization. Rolling back malaria. 1999; Chapter 4. Web: http://www.malaria.org.za/lsdi/Overview/Malaria and Development/malaria and-development.html; date of access: March 23, 2001.
2. Watt, J. M.; Breyer-Brandwijk, M. G. *The Medicinal and Poisonous Plants of Southern and Eastern Africa*. 2nd ed.; E. & S. Livingstone: Edinburgh, 1962; p 890.
3. Gessler, M. C.; Msuya, D. E.; Nkunya, M. H. H.; Mwasumbi, L. B.; Schär, A.; Heinrich, M.; Tanner, M. *J. Ethnopharmacol*. **1995**, *48*, 131.
4. Kamuhabwa, A.; Nshimo, C.; De Witte, P. *J. Ethnopharmacol*. **2000**, *70*, 143.
5. Weenen, H.; Nkunya, M. H. H.; Bray, D. H.; Mwasumbi, L. B.; Kinabo, L. S.; Kilimali, V. A. E. B. *Planta Med*. **1990**, *56*, 368.
6. Garo, E.; Maillard, M.; Hostettmann, K.; Stoeckli-Evans, H.; Mavi, S. *Helv. Chim. Acta* **1997**, *80*, 538.
7. Lee, I.; Shamon, L. A.; Chai, H.; Chagwedera, T. E.; Besterman, J. M.; Farnsworth, N. R.; Cordell, G. A.; Pezzuto, J. M.; Kinghorn, A. D. *Chem. Biol. Int*. **1996**, *99*, 193.
8. Jensen, J. B.; Trager, W. *J. Parasitol*. **1977**, *63*, 883.
9. Lambros, C.; Vandenberg, J. P. *J. Parasitol*. **1979**, *65*, 418.
10. Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother*. **1979**, *16*, 710.
11. Mosmann, T. *J. Immunol*. **1983**, *65*, 55.
12. Carmichael, J.; Degraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res*. **1987**, *47*, 936.
13. Melting points were determined on a Shimadzu DSC-50 apparatus. The ^{13}C and ^{1}H spectra were recorded on a Varian Gemini-300 spectrometer in a 1 Tesla magnetic field at a frequency of 300.075 MHz for ^{1}H and 75.462 MHz for ^{13}C spectra. All the chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS; $\delta = 0$). A Nicolet Nexus 470 FT-IR spectrometer was used to record IR spectra (in cm^{-1}) with the use of KBr pellets. The mass spectra were recorded on an analytical Varian VG 7070E mass spectrometer using electron impact at 70 eV as ionisation technique. NMR samples were dissolved in deutirated chloroform ($CDCl_3$). The following abbreviations are used to describe multiplicity of ^{1}H signals: s = singulet, d = doublet, m = multiplet. Combinations of these abbreviations indicate corresponding multiplicity of the signals. All R_f values were determined in petroleum ether/dichloromethane /ethyl acetate/ethanol 10:2:2:1.

Compound 1, 10-hydroxy-13-methoxy-9-methyl-15-oxo-20-norkaur-16-en-18-oic acid γ-lactone. White crystals; $C_{21}H_{28}O_4$; mp 152.84 °C; R_f 0.43; HRMS: 344.1989; *m*/*z* (%): 344.1989 M^+ (31), 316.893 (7), 300.2099 (21), 268.1831 (6), 241.1337 (10), 180.9923 (17), 130.9931 (31), 118.999 (27), 91.0545 (10), 68.9951 (100); ν_{max}: 2995.07, 2968.42, 2874.77, 1764.32, 1721.05, 1642.03, 1114.37, 937.11, δ_H: 1.01 (s, 3H, CH_3-19), 1.19 (s, 3H, CH_3-20), 1.31.83 (m, 6H, H-1,2,3,6,7,11), 2.11 (d, 1H, $J = 11.68$ Hz, H-14/12), 2.42 (dd, 1H, $J = 4.29$, 14.18 Hz; H-5), 3.16 (s, 3H, OCH_3-21), 5.27 (d, 1H, $J = 0.93$ Hz, H-17b), 6.04 (d, 1H, $J = 0.9$ Hz, H-17a); δ_C: 16.799 (C19), 17.945 (C6), 18.290 (C20), 19.765 (C2), 25.240 (C7), 30.896 (C1), 31.431 (C11), 34.453 (C12/14), 35.095 (C3), 40.376 (C14/12), 42.861 (C9), 47.252 (C4), 49.927 (C21), 51.642 (C5), 54.176 (C8), 79.588 (C13), 87.116 (C10), 116.357 (C17), 147.610 (C16), 180.309 (C18), 208.141 (C15).

Compound 2, 10,13-dihydroxy-9-methyl-15-oxo-20-norkaur-16-en-18-oic acid γ-lactone. White crystals; $C_{20}H_{26}O_4$; mp 202.00 °C; R_f 0.11; HRMS: 330.1833; *m*/*z*; (%): 330.1833 M^+ (24), 292.9824 (5), 286.1920 (17), 271.1718 (8), 242.9856 (6), 230.9856 (9), 180.988 (22), 168.988 (17), 130.9920 (32), 99.9936 (7), 68.9952 (100), 41.0392 (9); ν_{max}: 3415.06, 2933.74, 1763.49, 1720.02, 1112.45, 939.08; δ_H: 1.04 (s, 3H, CH_3-19), 1.22 (s, 3H, CH_3-20), 1.351.86 (m, 6H, H-1,2,3,6,7,11), 2.07 (m, 1H, H-12/14), 2.27 (d, 1H, $J = 11.84$ Hz, H-14/12), 2.45 (dd, 1H, $J = 4.29$, 14.31 Hz, H-5), 5.42 (s, 1H, H-17b), 6.01 (s, 1H, H-17a); δ_C: 16.869 (C19), 17.957 (C6), 18.389 (C20), 19.831 (C2), 25.040 (C7), 30.942 (C1), 31.885 (C11), 35.144 (C3), 36.014 (C12/14), 42.678 (C9), 46.638 (C14/12), 47.406 (C4), 51.777 (C5), 54.498 (C8), 75.056 (C13), 87.391 (C10), 115.581 (C17), 151.432 (C16), 180.695 (C18), 208.401 (C15).

Compound 3, 10-hydroxy-9-methyl-15-oxo-20-norkaur-16-en-18-oic acid γ-lactone. Colourless crystals; $C_{20}H_{26}O_3$; mp 160.90 °C; R_f 0.53; HRMS: 314.1888; *m*/*z*; (%): 314.1888 M^+ (41), 280.9824 (8), 271.1440 (38), 255.1793 (20), 242.9856 (6), 218.9856 (10), 199.1344 (8), 180.9888 (25), 168.9888 (18), 159.1120 (6), 130.9920 (34), 105.0708 (9), 91.0561 (14), 68.9952 (100), 41.0395 (15); ν_{max}: 2985.71, 2946.53, 2873.58, 1759.50, 1719.95, 1645.08, 1203.75, 1137.18, 935.3; δ_H: 1.03 (s, 3H, CH_3-19), 1.22 (s, 3H, CH_3-20), 1.381.88 (m, 4H, H-2,3,6,7), 1.443 (dd, 1H, $J = 0.07$, 0.017 Hz, H-11), 1.93 (dd, 1H, $J = 0.007$, 0.015 Hz, H-1), 2.11 (d, 1H, $J = 12.49$ Hz, H-14), 2.46 (dd, 1H, $J = 4.06$, 14.12 Hz, H-5), 2.84 (m, 1H, H-13), 5.18 (d, 1H, $J = 0.96$ Hz, H-17b), 5.90 (d, 1H, $J = 1.07$ Hz, H-17a); δ_C: 16.845 (C19), 17.964 (C6), 18.374 (C20), 19.857 (C2), 25.439 (C7), 29.188 (C14/12), 29.925 (C12), 30.820 (C1), 35.205 (C3), 37.345 (C13), 38.888 (C11), 43.414 (C9), 47.360 (C4), 51.87 (C5), 51.904 (C8), 87.667 (C10), 115.049 (C17), 149.099 (C16), 180.621 (C18), 210.781 (C15).