AD_____

Award Number: DAMD17-99-2-9027

TITLE: Drug Development and Conservation of Biodiversity in West and Central Africa

PRINCIPAL INVESTIGATOR: Cyrus J. Bacchi, Ph.D.

CONTRACTING ORGANIZATION: Pace University
New York, NY 10038-1502

REPORT DATE: May 2004

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20040907 014

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	May 2004	Final (1 May 1999 - 30 Apr 2004)

4. TITLE AND SUBTITLE

Drug Development and Conservation of Biodiversity in West and Central Africa

5. FUNDING NUMBERS

DAMD17-99-2-9027

6. AUTHOR(S)

Cyrus J. Bacchi, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Pace University
New York, NY 10038-1502

E-Mail: cbacchi@pace.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

This is the final report of associated project (AP #3) of the International Cooperative Biodiversity Group (ICBG) Program, which was headed by the Walter Reed Army Institute for Research (WRAIR). The project involves screening extracts of medicinal plants from Central and West Africa for growth-inhibitory activity vs. human and veterinary pathogenic African trypanosomes and trichomonads. Both drug-sensitive and drug-refractory isolates of each group were used. During this period approximately 281 extracts were received from WRAIR and the University of Dschang, Cameroon (AP #2). Approximately 2600 individual assays were done for trypanosomes to obtain IC_{50} values or growth inhibition data for 253 extracts. An additional 47 extracts were screened vs. trichomonads, comprising ~ 300 assays, and minimal inhibitory concentration (MIC) values obtained for all with at least one isolate. Forty-two extracts had IC_{50} values of ≤ 1.0 μg/ml for trypanosomes, and 38 had MIC values of ≤ 0.6 mg/ml for one or more of the trichomonad isolates. Some of the plant genera providing highly active material vs. trypanosomes were: *Premma, Cassia, Guarea, Melian, Goyania, Culcasis, Hyptis, Cassytha, Holarrhena, Jatropha, Combretum, Renealima, Boerrhavia* and *Erythrina.* Among the most active extracts vs. trichomonads were those from: *Aspilia, Combretum, Enantia, Hoslundia, Mormodica, Phyllanthus, Cleistopholis, Mitracarpus,* and *Draecaena.*

14. SUBJECT TERMS

African plants, protozoan parasites, phytomedicine

15. NUMBER OF PAGES

26

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Cover.....

SF 298.....

Introduction.....3

Body.....4–5

Key Research Accomplishments.....6

Reportable Outcomes.....6

Conclusions.....7

References.....8–9

Appendices.....10

I. Introduction

This project concerns drug discovery and development through ethnobotanical leads to agents in growth screens against African trypanosomes and pathogenic trichomonads. These diseases are of significant concern in continental Africa and elsewhere in developing countries, particularly those in which AIDS is prevalent. In some cases disease is cosmopolitan (*Trichomonas vaginalis*), in some areas, new human epidemics have begun (African human trypanosomiasis, e.g, *Trypanosoma rhodesiense*) and in some instances diseases pose significant threats to livestock and their breeding (*Trypanosoma brucei* and *Tritrichomonas foetus*). This ICBG program is under the direction of WRAIR and concerns plant extracts from Central and West Africa. These are supplied by ethnobotanists and chemists at WRAIR and through the Department of Chemistry, University of Dschang (Cameroon, AP#2).

Human African trypanosomiasis is endemic over 10 million square kilometers of sub-Saharan African, affecting human as well as all domesticated livestock (WHO, 1995). Recently, the number of new human cases of sleeping sickness has escalated from ~ 25,000/year to 500,000/year and an incidence of veterinary sickness of 300,000 cases (WHO 2001, van Nieuwenhove et al. 2001). These estimates are most likely low, based on civil unrest and lack of local medical surveillance (F. Kuzoe, pers. Commun.). The major drugs for human disease, pentamidine and melarsorpol (Arsobal®) have been in use > 50 years. These agents, particularly melarsoprol, have associated CNS and other toxicity, and their continued used has led to an increased incidence of resistance (van Nieuwenhove, 1992; Kuzoe 1993; Wery 1994). Melarsoprol remains the only drug in common use for late stage CNS disease (Burri and Keiger 2001; Legros et al., 1999).

Trichomonas vaginalis is a sexually transmitted human pathogen of the urogenital tract. It affects the vaginal epithelium causing severe irritation. Trichomonisais one of the most prevalent STDs in the western world (Lossik, 1989; Hammill 1989). Recent evidence suggests a high incidence rate between cervical cancer and trichomoniasis. In the United States alone, there are ~ 3 million reported cases (Hook 1999). Studies in developing countries and cosmopolitan areas of large cities indicate the concurrent presence of *T. vaginalis* infection and exposure to HIV-1 in semen is an important additional risk for contraction of AIDS (Laga et al., 1993; Sorvillo and Kerndt 1998; Hook 1999; Jackson et. al., 1997). The single major treatment for human trichomoniasis is a 5-nitroimidazole, metronidazole (Fagyl®), which has been in continuous extensive use since 1955 in the United States and Europe; drug-resistant strains are becoming more prevalent (Meingassner and Thurner 1979; Voolman and Boreham 1993; Wong et. al., 1990). It is potentially mutagenic, based on ability to form free radicals and is not given to pregnant women (Lossick 1989). At present there is no alternative therapy for metronidazole-refractory disease or for pregnant women.

Trichomonas foetus is the agent of bovine trichomoniasis, causing reproductive failure. Parasites are spread by infected bulls and cause abortion of the fetus. In some cases, the cow is permanently sterilized. There is no satisfactory treatment of infected bulls since metronidazole kills the rumen flora. Unless the bull is valuable, it is usually destroyed (Levine 1985).

II. Body

1. Methods

a) African trypanosomes. *In vitro* screens with bloodstream form trypanosomes are set up in 24 well plates using duplicate wells of four extract concentrations each (in HMI medium: Hirumi & Hirumi, 1989) plus full-growth controls, as detailed in Bacchi et al. (1997). Initial wide concentration curves were followed by narrow-ranging curves to determine IC_{50} values. Strains of trypanosomes used were: *Trypanosoma brucei*, Lab 110 EATRO (veterinary parasite); *Trypanosoma rhodesiense* KETRI 243 (human isolate), *T. rhodesiense* 243As 10-3 (clone of KETRI 243 highly resistant to melarsoprol and pentamidine). Usually 2 -3 growth curves are necessary to determine an IC_{50} value. Each experiment is incubated at 37°C for 72 h in 5% CO_2.

b) Trichomonads. The method used was the minimal inhibitory concentration (MIC) assay developed by Meingassner et al. (1978). Strains used were *T. vaginalis* C1-NIH (ATTC 30001) and a metronidazole-resistant strain, CDC-085 (ATCC 50143) and KV-1, a *Tritrichomonas foetus* extract. These are incubated aerobically in 96 well plates with triplicate serial dilutions of each extract, and counted microscopically at 24 and 48 h.

c) *In vivo* studies. For African trypanosomes, extracts having IC_{50} values of ≤ 20 μg/ml were tested in a *T. brucei* Lab 110 EATRO mouse model infection (Bacchi et al., 1990). Mice (10–25 g) were infected with 5×10^5 trypanosomes and treatment was begun 24 h later. Since all extracts were solubilized in 50% DMSO, extracts were diluted in this solvent to achieve correct concentrations. Mice (3 per dose point) were dosed once daily for three days, by the intraperitoneal route. Animals surviving > 30 days with no evidence of parasites in blood smears are considered cured.

2. Results

a) African trypanosomes. Over the course of this grant period, a total of 216 extracts were received from Dr. Chris Okunji of WRAIR and 65 from Drs. Apollinaire Tsopmo and Pierre Tane (University of Dschang, Cameroon AP#2). A total of 253 of the 281 extracts were tested vs three strains of trypanosomes, and assays for the remaining 28 extracts are in progress.

During the grant period, 42 of the WRAIR extracts and three AP #2 extracts had IC_{50} values of ≤ 1 μg/ml for trypanosomes. Many more had IC_{50} values of ≤ 20 μg/ml. With the exception of ~ 30 WRAIR extracts, all were tested vs. the 3 strains of trypanosomes (Tables 2 and 3). Difficulties in culture of *T. rhodesiense* 243 and 243 As 10-3 prevented testing with these strains during the past several months. The 48 most active extracts are listed in Table 4. Differences in the activity with relation to parts of the plant extracted and type of solvent used were evident in this study: e.g., SU 1878 *Phyllanthus amerus* (CH_2Cl_2) had higher activity than SU 1879 (aqueous); SU 1891 *Uvaria chamae* (aqueous) was more active than SU 1889 (CH_2Cl_2); SU 2164 *Holarrhena floribunda* (leaves-methanol) was more active than SU 2165 (leaves-aqueous).

A total of 65 extracts were received from the group at the University of Dschang (Table 3). Of these, 15 are in the early stage of investigation, with preliminary data or inconclusive results. However, of the remaining 50, one had IC_{50} values ≤ 20 μg/ml, and 4 were < 5 μg/ml

and one (ASP) was < 1 μg/ml. Data concerning the latter extract and others from *Afromomum* has been published (Kamnaing et al., 2003).

In vivo studies. During the 5-year grant period, a total of 43 extracts were tested vs. a *T. brucei* mouse model infection. These included some extracts supplied and tested *in vitro* prior to this grant period, but which were tested *in vivo* during the current period. Extracts were diluted with DMSO:H_2O and administered i.p. once daily at 1, 5, 10, and 25 mg/kg/day for 3 days. Although none of the extracts was curative or prolonged life as compared to infected untreated controls, the extracts were relatively insoluble in aqueous solutions, and the DMSO concentration limited the largest dose possible to 25 mg/kg. A partial listing of extracts tested *in vivo* is given in Table 4. Some extracts listed as very active *in vitro* could not be tested *in vivo* because of insufficient supplies. Future studies should attempt to use more purified material, in powder-form if possible.

b) Trichomonads. A total of 47 plant extracts were screened vs. trichomonad isolates: *Trichomonas vaginalis*, metronidazole-sensitive (C1-NIH; ATCC 3001), and –resistant (CDC-085; ATCC 50143) and the veterinary parasite, *Tritrichomonas foetus* (KV-1).

All of the 47 extracts were tested, many vs. all 3 isolates. Later studies were limited to the *T. foetus* isolate, since the *T. vaginalis* isolates could not be maintained reliably in culture. Initial studies found a number of extracts yielding MIC values ≤ 0.1 mg/ml for all 3 isolates (Table 5). These included SU-1458, 1460, 1461, 1462, 1464, 1752, and 1763. Note differences in MIC values for metronidazole-sensitive and –refractory isolates. Another eight isolates obtained during 2001-2003, were tested vs. the drug-sensitive and –resistant *T. vaginalis* isolates (Table 6). Many of these extracts were oils which made them difficult to work with. Of this group of compounds, SU 1863, 1870, 1873, and 1877 had MIC values of < 1 mg/ml, and are considered worthy of future study. Extracts SU1866 – 1902 were screened for activity vs. *T. foetus* and SU- 1874, 1877, 1879, 1895, and 1896 found to have MIC values of ≤ 1 mg/ml (Table 7). The 14 plants whose extracts proved most active vs. the trichomonads are listed in Table 8. Although some of these extracts were significant inhibitors of trypanosome growth, there appeared to be a degree of specificity for trichomonads associated with most extracts: 10 of 14 extracts having the most activity vs. trichomonads were not significant inhibitors of trypanosome growth.

c) Training. Part of the function of Haskins Laboratories at Pace University is to train undergraduates in research techniques. In this 5-year reporting period, a total of 10 undergraduates participated in this research, and presented papers on their work at the Dyson College Society of Fellows Workshop (Arts and Sciences Honor Society). Many other undergraduates have similarly taken part in this work over the past 10 years. In addition, the Woodrow Wilson Foundation, joint with NSF, annually holds a two week High School Science Teacher Summer Training Institute at Pace University. This program features the ecosystem as a major topic and the students are given lectures and laboratory demonstrations concerning biodiversity, conservation of ecosystem resources and the activities of the ICBG program.

Issues of concern are resolving culture problems with *T. vaginalis* extracts, and the identification of active agents in those plant extracts which are highly growth inhibitory vs. trichomonads and trypanosomes.

III. Key Research Accomplishments

- Identification of a total of 14 plants whose extracts were significantly inhibitory to trypanosome growth, with IC_{50} value of ≤ 1 μg/ml. These are listed in Table 4.

- Indication of specificity with respect to solvent used, indicating selective extraction of an active agent, e.g., *Holarrhena floribunda* leaf methanol extract (SU 2164) was far more active than the CH_2Cl_2 extract (SU 2165) vs. African trypanosomes.

- Proof of selective specificity of extracts with respect to trypanosomes and trichomonads was obtained, indicating growth inhibition was not due to general cytotoxicity. In general, the most active extracts vs. trypanosomes were not the most active vs. trichomonads.

- Despite the *in vitro* activity of many extracts vs. trypanosomes, activity of crude and partially purified extracts in the *in vivo* mouse model was not demonstrated. This, in large part, was the need to use DMSO as a solubilizing agent, limiting dosages to mice to 25 mg/kg. Additional studies are needed to assess *in vivo* activity, using powdered extracts in place of oils or waxy materials.

IV. Reportable outcomes

Kammnaing, P., Tsopmo, P., Tanifum, E.A., Tane, P., Ayafor, J.F., Sterner, O., Rattendi, D., Iwu, M.M., Schuster, C., and Bacchi, C.J. 2003. Diarylheptanoids from *Afromomum letestuianam* K. Schum (Zingiberaceae). J. Natural Products, 66:364-367.

Tsopmo, P., Kammnaing, P., Ngamga, D., Tane, P., Ayafor, J.F., Sterner, O., Rattendi, D., Iwu, M.M., Schuster, C., and Bacchi, C.J. Antitrypanosomal alkaloids from *Xymalos monospora.* J. Natural Products (under review).

Okunji, C., Tane, P., Ngamga, D., Abegaz, B.M., Bacchi, C.J., and Iwu, M.M. 2004. Abstract: International Congress on National Products Research. July 31 – August 4, 2004, Phoenix AZ. *In vitro* antiprotozoal activity of ethnomedically selected members of the family *Fabaceae.*

U.S. Patent Application Serial No. 09/382,128
Filing Date: August 24, 1999
For: Antifungal and Antiparasitic Compounds
By: J.E. Jackson, M.M. Iwu, C.O. Okunji, C. Bacchi, J.D. Tally, Jr., and J.F. Ayafor.

U.S. Patent Application Serial No. 09/428,203
Filing Date: October 27, 1999
For: Plant-derived Antiparasitic Antifungal Compounds and Methods of Extracting the Compound
By: C.O. Okunji, .J.E.. Jackson, M.M. Iwu, C. Bacchi, J.D. Tally, Jr., and J.F. Ayafor.

V. Conclusion

Significant and selective growth inhibitory activity of plant extracts vs. pathogenic protozoa was obtained in this study. Selectively for African trypanosomes or pathogenic trichomonads was clearly shown as 12 of 16 extracts inhibitory to trichomonads were not significantly active vs. trypanosomes (Table 8). Some of the most active extracts vs. trypanosomes may have a common link: they are from the family Fabaceae and may be source of cryptoleptins, e.g., *Erythrina* (SU 1760), *Cryptolepis* (SU 1754), *Plantex* (SU 1757), and *Fagara* (SU 1758). (M. Iwu, pers. commun.). Related studies have indicated that other members of the family *Facaceac* were highly active inhibitors of *Plasmodium falciparum* growth. The seeds of one plant, *Millettia griffoniana* were found to contain two new isoflavonoid compounds (7-ethoxyebenosin, griffianone-F) and two known compounds (7-methoxy-8(3,3-diethyl-allyl)isoflavone and calopogonium isoflavone (Okunji et al., 2004). Although the ICBG grant has ended, we have received purified fractions of some of the extracts found to be active vs. trypanosomes and trichomonads, and intend to test them *in vitro* to determine whether they contain the active growth inhibitory compounds.

Economically, this is an important program for Nigeria and Cameroon: both are endemic for human and veterinary trypanosomiasis, while STD infections, including *T. vaginalis* and bovine trichomoniasis are a significant source of human suffering and an economic drain. There is an urgent need for new and inexpensive drugs for trypanosomiasis. Treatment of human trichomoniasis depends solely on metronidazole, and there is no agent currently available for bovine trichomoniasis [metronidazole kills the microbial flora of the rumen and cannot be given to cattle]. Development of anti-protozoal agents from local plants would be a major factor in the well-being of these populations and a boost to local and national economics.

<u>Personnel Receiving Grant Pay</u> (All were paid part-time)

Lakshman Mazumder	–	Animal Laboratory Technician
Alicja Bierut	–	Technician
Jenny Gallardo	–	Technician
Elvis Rosario	–	Technician
Marisol Torres	–	Technical Aide

VI. References

Bacchi CJ, Nathan HC, Livingston T, Valladares G, Saric M, Sayer PD, Njogu AR, Clarkson Jr, AB. 1990. Differential susceptibility of DL-α-difluoromethylornithine in clinical isolates of *Trypanosoma brucei rhodesiense*. Antimicrob Agents Chemother 34:1183-1188.

Bacchi CJ, Sanabria K, Spiess AJ, Vargas M, Marasco Jr, CJ, Jimenez LM, Goldberg B, Sufrin, JR 1997. *In vitro* efficacies of 5'-methylthioadenosine analogs as trypanocides. Antimicrob Agents Chemother 41:2108-2112.

Burri C, Keiser J. 2001. Pharmacokinetic investigations on patients from Northern Angola refractory to melarsoprol treatment. Trop Med Int Health 6:412-420.

Hammill MA. 1989. *Trichomonas vaginalis*. Obstet. Gynecol. Clin. North Am. 16:531-540.

Hirumi H, Hirumi K. 1989. Continuous cultivation of *Trypanosoma brucei* bloodstream forms in a medium containing a low concentration of serum protein without feeder layer cells. J Parasitol. 75:985-989.

Hook III, EW. 1999. *Trichomonas vaginalis* – no longer a minor STD. Sex. Trans. Dis. 26:388-389.

Jackson DJ, Rakwar JP, Bwayo JJ, Kreiss JK, Moses S. 1997. Urethral *Trichomonas vaginalis* infection and HIV-1 transmission. Lancet. 350:1076.

Kammnaing, P., Tsopmo, P., Tanifum, E.A., Tane, P., Ayafor, J.F., Sterner, O., Rattendi, D., Iwu, M.M., Schuster, C., and Bacchi, C.J. 2003. Diarylheptanoids from *Afromomum letestuianam* K. Schum (Zingiberaceae). J. Natural Products, 66:364-367.

Kuzoe F. 1993. Current situation of African trypanosomiasis. Acta Tropica 54:153-162.

Laga M, Monoka A, Kivuvu M, et al. 1993. Non-ulcerative sexually transmitted diseases as risk factors for HIV-1 transmission in women: results from a cohort study. AIDS 7:95-101.

Legros D, Fournier C, EtchegorryMG, Maiso F, Szumilin E. 1999. Therapeutic failure of melarsoprol among patients treated for late stage of *T. b. gambiense* human African trypanosomiasis in Uganda. Bull Soc Path Exot. 92:171-172.

Levine ND. 1985. Veterinary Protozoology. pp 59-79. Iowa State Univ. Press, Ames.

Lossick JG. 1989. "Therapy of urogenical trichomoniasis", in Trichomonads Parasitc in Humans. Honigberg BM (ed.), Springer-Verlag, New York, pp 324-341.

Meingassner JG, Mieth H, Czok R, Lindmark DG, Muller M. 1978. Assay conditions and demonstration of nitroimidazole-resistance in *Trichomonas foetus*. Antimicrob Agents Chemother. 13:1-3.

Meingassner JG, Thurner J. 1979. Strain of *Trichomonas vaginalis* resistant to metronidazole and other 5-metronidazoles. Antimicrob Agents Chemother. 15:254-258.

Okunji, C., Tane, P., Ngamga, D., Abegaz, B.M., Bacchi, C.J., and Iwu, M.M. 2004. Abstract: International Congress on National Products Research. July 31 – August 4, 2004, Phoenix AZ. *In vitro* antiprotozoal activity of ethnomedically selected members of the family *Fabaceae.*

Sorvillo F, Kerndt P. 1998. *Trichomonas vaginalis* and amplification of HIV-1 transmission. Lancet. 351:213-214.

van Nieuwenhove S. 1992. Advances in sleeping sickness therapy. Ann Soc Belg Med Trop. 72:39-51.

van Nieuwenhove S. 2001. Sleeping sickness resurgence in the DRC: the past decade. Trop Med Int Health 6:335-341.

Voolman T, Boreham P. 1993. Metronidazole-resistant *Trichomonas vaginalis* in Brisbane. Med. J Australia. 159:490.

Wery M. 1994. Drugs used in the treatment of sleeping sickness (human African trypanosomiasis: HAT). Int J Antimicrob Agents 4:227-238.

Wong A, Wilson PD, Chew TA. 1990. Providone-iodine in the treatment of metronidazole-resistant *Trichomo nas vaginalis*. Australia-New Zealand J Obstet Gynecol. 30:169-171.

World Health Organization. 2001. World Health Organization and Aventis announce a major initiative to step up efforts against sleeping sickness. WHO Press Release. 23.

World Health Organization. 1995. Tropical Disease Research Twelfth Programme Report. World Health Organization, Geneva.

Table 1. Compounds tested vs. animal or veterinary-parasitic African trypanosomes and trichomonads during the course of this proposal.

Month/Year	African Trypanosomes		Trichomonads
	WRAIR	AP#2 Cameroon	WRAIR
6/99 – 5/00	24	14	17
6/00 – 5/01	40	6	12
6/01 – 5/02	39	8	10
6/02 – 5/03	73	8	8
6/03 – 5/04	40*	29**	0
	216	65	47

*28 still in progress
** 15 still in progress

Table 2. IC_{50} values for plant extracts received from WRAIR (Jan. 2003). Compounds were tested vs. trypanosome isolates grown in blood forms in HMI-18 medium containing 20% fetal bovine serum. Coulter counts were made daily and IC_{50} values determined after 48 h as described in the text. (Data thru May 2004).

	IC_{50} (µg/ml)		
	Lab110 EATRO	KETRI 243	KETRI 243 As10-3
SU-2140	2.8	10	8.7
SU-2141	44% @ 500 µg/ml	+ @ 500 µg/ml	-
SU-2142	25.5	21	55
SU-2143	330	500	11% @ 500 µg/ml
SU-2144	67	50	43
SU-2145	6.6	5.5	48% @ 25 µg/ml
SU-2146	28.5	16.5	34
SU-2147	250	500	96
SU-2148	4.0	1.575	6.2
SU-2149	12	19.25	22.5
SU-2150	55	112.5	125
SU-2151	0.525	0.525	1.25
SU-2152	71	32	22.0
SU-2153	27.5	22.5	88
SU-2154	66	71	100
SU-2155	9.3	22.5	64
SU-2156	5.8	77	12.5
SU-2157	0.235	19.5	3.85
SU-2158	0.1	3.7	2.45
SU-2159	7.5	12.25	14.0
SU-2160	25% @ 500 µg/ml	44% @ 500 µg/ml	-
SU-2161	43% @ 500 µg/ml	44% @ 500 µg/ml	-
SU-2162	4.6	61	51.5
SU-2163	5.0	10	22.0
SU-2164	0.5	6.7	6.8
SU-2165	3.8	16.5	27.0
SU-2166	5.0	20.0	18.75
SU-2167	0.195	16.5	17.0
SU-2168	20% @ 500 µg/ml	41% @ 250 µg/ml	157.5
SU-2169	11% @ 500 µg/ml	80	71.0
SU-2170	17% @ 500 µg/ml	500	31% @ 500 µg/ml
SU-2171	42.5	55.5	-
SU-2172	145	75.6	-
SU-2175	50	-	-
SU-2176	23.5	> 100 µg/ml	18.75
SU-2177	19.0	19.25	19.50
SU-2178	14.25	19.00	17.00
SU-2179	14.75	13.75	15.00
SU-2180	9.5	> 50 µg/ml	> 25 µg/ml

Table 2 (continued)

	IC_{50} (μg/ml)		
	Lab110 EATRO	KETRI 243	KETRI 243 As10-3
SU-2181	59	-	-
SU-2192	2.2	21	8.9
SU-2194	16.5	7.6	16.5
SU-2195	71	-	-
SU-2196	120	-	-
SU-2197	18.5	21.5	> 50 μg/ml
SU-2198	24.0	28.0	35.0
SU-2200	0.92	1.325	1.7
SU-2201	77	-	-
SU-2202	290	-	-
SU-2203	37	24	6.5
SU-2204	70	-	-
SU-2205	135	-	-
SU-2206	6.75	6.7	8.0
SU-2207	28.75	2.4	3.4
SU-2208	265	-	-
SU-2209	16	44% @ 50 μg/ml	22
SU-2210	25	23	22.25
SU-2211	315	-	-
SU-2212	63.5	-	-
SU-2213	35% @ 500 μg/ml	-	-
SU-2214	89	-	-
SU-2215	2.85	37% @ 100 μg/ml	39% @ 100 μg/ml
SU-2216	165	-	-
SU-2217	110	-	-
SU-2218	63	-	-
SU-2219	84	-	-
SU-2220	177.5	-	-
SU-2250	[0.05 – 1.0]	-	-
SU-2251	9.35	-	-
SU-2252	[0.5 – 10]	-	-
SU-2253	[0.05 – 1.0]	-	-
SU-2254*	-	-	-
SU-2255	-	-	-
SU-2256	-	-	-
SU-2257	-	-	-
SU-2258	-	-	-
SU-2259	-	-	-
SU-2260	-	-	-
SU-2261	-	-	-
SU-2496	-	-	-

[ ] = IC_{50} value should fall in this range
* SU 22254 – 2496 in progress

Table 2 (continued)

	IC_{50} (μg/ml)		
	Lab110 EATRO	KETRI 243	KETRI 243 As10-3
SU-2497*	-	-	-
SU-2499	-	-	-
SU-2500	-	-	-
SU-2501	-	-	-
SU-2502	-	-	-
SU-2516	-	-	-
SU-2681	-	-	-
Melarsoprol	0.0075	0.016	0.016
Pentamidine	0.0008	0.00098	0.00075

*SU 2497 – 2681 – in progress

Table 3. Activity of University of Dschang (AP #2) extracts vs. African trypanosomes *in vitro*. Data to May 2004.

	IC_{50} (µg/ml)		
		KETRI	
	Lab110 EATRO	**243**	**243 As 10-3**
ASP	0.5	1.5	3.9
ASS_2	28	26.5	130
ASS_4	30.5	16	51
ASS_5	55	50.1	56
TZM_{1A}	21.5	18	15.9
TZM_1	2.35	22.5	17
TZM_4	4.45	2.1	1.85
TZM_4 HCl	3.59	3.59	1.80
TZM_5	25	43.5	-
NMG-2	125	-	-
NMG-3	8.65	40% @ 25 µg/ml	33% @ 25 µg/ml
NMG-5	16.0	16.75	26
NMG-6	1% @500 µg/ml	-	-
GLY	2.3	[1 – 25]	6.2
MG1	230	[100 – 500]	1% @500 µg/ml
MG2*	+ @500 µg/ml	-	-
MG3	100	161	[50 – 250]
AVX-1	37	62	[10 – 100]
AVX-2	31% @ 25 µg/ml	-	-
TPA/H*	225	-	-
PAY-1	[10 – 500]	-	-
PAY-2	30% @ 50 µg/ml	-	-
PAY-3*	64	-	-
TAX-1*	6.0	[1 – 25]	[1 – 25]
TAX-2*	28.3	-	-
TAX-3*	76	-	-
CPF	155	[10 – 250]	65.5
NMG-1	-	-	-
NMG-2	-	-	-
NMG-3	-	-	-
NMG-4*	165	-	-
NMG-5	-	-	-
NMG-6	-	-	-
CPB-2	42.5	[10 – 100]	+ @ 50 µg/ml
CPB-3*	42% @50 µg/ml	-	-
CPB-4	[10 – 500]	-	-
CPB-8	[10 – 500]	-	-
GBF-1*	10.25	-	-
GBF-2	-	-	-
GBF-3*	[0.5 – 50]	-	-
GBF-5*	-	-	-
GBF-6*	6.4	-	-

* Approximate µg/ml values – samples could not be accurately weighed because of viscosity.
[ ] = IC_{50} value should fall in this range

Table 4. WRAIR extracts, received through April 2003 having significant activity $IC_{50} \geq 20$ µg/ml vs. African trypanosomes *in vitro: T. b. brucei* Lab 110 EATRO. Those extracts tested in the mouse model infections are indicated.

Extract	Plant	Extract	Plant
SU 1460** [x]	*Afromomum aulocacarpus*	SU 1871 [x]	*Combretum dulchipetalim* (Aq)
SU 1461 [x]	*Dracaena mannii*	SU 1872** [x]	*Cryptolepis sanguinolenta*
SU 1754**	*Cryptolepis sanguinolenta*	SU 1873 [x]	*Enantia chlorontha*
SU 1757*	*Plantex vellous*	SU 1874* [x]	*Hoslundia opposita*
SU 1758*	*Fagara lemairei (stem bark)*	SU 1875 [x]	*Icacina trichanta (tuber)*
SU 1760*	*Erythrina senegalenis (root)*	SU 1878** [x]	*Phyllanthus amerus* (CH_2Cl_2)
SU 1767*	*Glossocalyx brevipes (alkaloid (fr.)*	SU 1879 [x]	*Phyllanthus amerus* (Aq.)
SU 1768** [x]	*Glossocalyx brevipes* (neutral fr.)	SU 1880**	*Pleiocarpa pycnantha*
SU 1769* [x]	*Dorsternia barteri*	SU 1881	*Scoparia dulcis*
SU 1863 [x]	*Aspilia Africana*	SU 1886 [x]	*Trimfetta tomentosa*
SU 1866 [x]	*Chamacrista mimosoides*	SU 1889 [x]	*Uvaria chamae* (CH_2Cl_2)
SU 1869 [x]	*Combretum dulchipetalum* (CH_2Cl_2)	SU 1891** [x]	*Uvaria chamae* (Aq.)
SU 1870 [x]	*Combretum dulchipetalum* (Aq)		

*Very active, $IC_{50} < 5$ µg/ml; **Most active, $IC_{50} \leq 1$ µg/ml.
[x]Tested *in vivo*

Table 4 (continued)

Extract	*Plant*	Extract	*Plant*
SU 2140* [x]	*Premma guadrifolia*	SU 2164** [x]	*Holarrhena floribunda* (leaves)
SU 2145 [x]	*Cassia siamea* (leaves/stems) (dry)	SU 2165* [x]	*Holarrhena floribunda* (leaves)
SU 2148* [x]	*Guarea thompsonli* (Stem bark)	SU 2166 [x]	*Jatropha curcas* (leaves)
SU 2149 [x]	*Guarea thompsonli* (Stem bark)	SU 2167** [x]	*Jatropha curcas* (stems)
SU 2151** [x]	*Melian excelsa* (Stem bark)	SU 2180	*Combretum dulchipetalum* (leaves)
SU 2156 [x]	*Goyania long pelara* (Leaves/stems)	SU2192*	*Renealmia porypus*
SU 2157** [x]	*Culcasis scanders* (whole plant)	SU 2200**	*Boerrhavia diffusa* (roots)
SU 2158* [x]	*Hyptis suaveolens* (leaves)	SU 2206*	*Ficus thonnigii*
SU 2159 [x]	*Hyptis suaveolens* (leaves)	SU 2215*	*Moringa olifera* (leaves/stems)
SU 2162* [x]	*Cassytha filiformis* (whole plant)	SU 2250**	*Hyptis suaveolens* (leaves) subfraction 1 – 36
SU 2163 [x]	*Cassytha filiformis* (whole plant)	SU 2253**	*Hyptis suaveolens* (leaves) subfraction 58-61

*Very active, $IC_{50} < 5$ μg/ml; **Most active, $IC_{50} \leq 1$ μg/ml.
[x] Tested *in vivo*

Table 5. Inhibition of Trichomonas growth by new primary plant extracts received in 1999 - 2000. The assay system used was the standard MIC (minimal inhibitory concentration) assay (Meingassner et al 1978) for Trichomonas in which serial dilutions were prepared in medium using sterile 96 well plates. Twelve dilutions were made, with a concentration range of 2.5 to 0.0012 mg/ml. Each well contained 10^4 organisms. Plates were incubated aerobically for 48h then examined. The MIC is defined as the minimum concentration of drug in which no motile organisms are visible after 48 h incubation. CI-NIH is metronidazole sensitive, CDC-085 is metronidazole-resistant and KV1 is the cattle parasite, *Tritrichomonas foetus*. Data expressed as MIC in mg/ml. ND, not determined.

		MIC		
Extract	**Origin**	**C1-NIH**	**CDC-085**	**KV1**
SU 1458	*Araliopsis tabouensis* AZ_2	0.2	0.4	0.4
SU 1460	*Afromonum aulocacarpus*	0.1	0.0015	0.1
SU 1461	*Dracaena mannii Mannispirostan A*	0.0125	0.006	0.05
SU 1462	*Napoleonaea imperialis* MEOH	0.1	-	0.4
SU 1463	*Pachypodanthium staudtii* CH_2Cl_2	0.80	ND	>0.80
SU 1464	*Glossocalyx brevipes* CH_2Cl_2	0.0125	0.0125	0.0125
SU 1465	*Enantia chlorantha* MeOH	0.80	0.10	>0.80
SU 1466	*Eupatorium odoratum* MEOH	0.4	0.4	0.4
SU 1467	*Cleistopholis patens* EtOH	>0.80	0.10	>0.80
SU 1468	*Leidobotrys staudii* CH_2Cl_2	0.40	ND	>0.80
SU 1469	*Ancistocladus barteri* ABSBM	0.40	ND	0.40
SU 1751	*Eupatorium adoratum*	0.3	0.6	-
SU 1752	*Eupatorium adoratum* MEOH	0.3	0.3	-
SU 1758	*Fagara lemairei*	0.6	-	-
SU 1759	*Fagara lemairei* MEOH	0.6	-	-
Su 1763	*Mitracarpus scaber* (Pet. Ether)	0.1	0.9	-
Metronidazole		0.003	0.40	0.003

Table 6. Activity of plant extracts vs. growth of *Trichomonas vaginalis in vitro*. Assays were performed in 200 □l wells in triplicate. Each extract was dissolved in DMSO and diluted with growth medium. Assays are read at 24 and 48 hours and MIC value determined by the lowest concentration of extract at which triplicate wells show no growth (Meingassner et al 1978). Strain 30001 is Metronidazole sensitive, strain 50143 is Metronidazole refractory.

	MIC (mg/ml)			
	ATCC 30001		ATCC 50143	
Extract	**24h**	**48h**	**24h**	**48h**
SU 1863	1.3	0.6	<1.3	0.6
SU 1864	1.3	<1.3	<1.3	<1.3
SU 1865	<1.3	<1.3	<1.3	<1.3
SU 1868	1.3	0.6	1.3	1.3
SU 1870	0.3	0.3	0.3	0.3
SU 1873	1.3	1.3	1.3	0.6
SU 1876	<1.3	1.3	<1.3	<1.3
SU 1877	<1.3	<1.3	<1.3	0.6
Metronidazole	0.005	0.003	0.60	0.40

Table 7. Activity of WRAIR extracts received 2001 - 2003 *in vitro* vs. *T. foetus* KV1. Methods as in Table 5.

	MIC (mg/ml)			MIC (mg/ml)	
Extract	24 h	48 h	Extract	24 h	48 h
SU 1863	1.3	–	SU 1884	1.3	1.3
SU 1864	>1.3	>1.3	SU 1887	>1.3	>1.3
SU 1865	>1.3	>1.3	SU 1889	1.3	1.3
SU 1866	1.3	1.3	SU 1890	>1.3	1.3
SU 1867	>1.3	>1.3	SU 1891	>1.3	1.3
SU 1869	>1.3	1.3	SU 1892	>1.3	>1.3
SU 1872	>1.3	1.3	SU 1893	1.3	1.3
SU 1873	1.3	–	SU 1894	>1.3	>1.3
SU 1874	0.6	0.3	SU 1895	0.63	0.63
SU 1875	>1.3	1.3	SU 1896	0.31	0.31
SU 1878	1.3	1.3	SU 1898	>1.3	–
SU 1879	1.3	0.6	SU 1899	1.3	1.3
SU 1880	>1.3	1.3	SU 1900	>1.3	>1.3
SU 1881	>1.3	1.3	SU 1901	>1.3	>1.3
SU 1882	1.3	1.3	SU 1902	1.3	1.3
SU 1883	>1.3	1.3	Metronidazole	–	0.003

Table 8. Most active WRAIR extracts vs. Trichomonads (MIC $\leq$ 0.6 mg/ml)

Lab Number	Plant Name	Extract
SU 1458	*Araliopsis tabouensis* AT7	-
SU 1460	*Afromomum aulocacarpus* AZ_2	-
SU 1461	*Dracaena mannii*	-
SU 1462	*Napoleonaea imperialis*	MEOH
SU 1464	*Glossocalyx brevipes*	CH_2Cl_2
SU 1751	*Eupatorium odoratum*	MEOH
SU 1752	*Eupatorium odoratum*	MEOH
SU 1763	*Mitracarpus scaber*	MEOH
SU 1863*	*Aspilia africana*	CH_2Cl_2
SU 1868	*Combretum dulchipetalim*	MeOH
SU 1870*	*Combretum dulchipetalim*	Aq.
SU 1873*	*Enantia chlorontha*	MeOH
SU 1874*	*Hoslundia opposita*	CH_2Cl_2
SU 1877	*Mormodica charanta*	CH_2Cl_2
SU 1879	*Phyllanthus amerus*	Aq.
SU 1895	*Cleistopholis patent*	CH_2Cl_2

* Also active vs. trypanosomes

2004 – International Congress on Natural Products Research
July 31 – August 4, 2004
Phoenix, Arizona USA

ABSTRACT SUBMISSION FORM

Corresponding author's name: Christopher Okunji	
Phone: 301-319 9762	Fax: 301-319 9449
E-mail address: **christopher.okunji@na.amedd.army.mil**	

Please underline preferred presentation format:
Oral Poster No Preference

Please underline one category that best describes the focus of your abstract:
Bioassay Method Development Biosynthesis/Biochemistry Biotechnology
Biotransformation/Metabolism Chemical Ecology Herbal Drugs/Nutraceuticals
Documentation of Plant Use Isolation/Structure Elucidation
Phytochemistry/Systematics Synthesis
Other (please specify):

Please underline the type of organism used in the research:
Bacteria Fungi Other microorganisms
Marine Macroorganisms Non-Marine Animals Plants
Other (please specify): ***Plasmodium falciparum, Trypanosoma b. brucei***

_☐ **Place an "X" in front of this box.** This abstract is submitted by an undergraduate or graduate researcher, and it should be considered for the ASP Student Poster Awards

In vitro Antiprotozoal Activity of Ethnomedically Selected members of the family Fabaceae.

Christopher Okunji[1,5], Pierre Tane[2], Dieudonné Ngamga[2], Berhanu M. Abegaz [3], Cyrus Bacchi[4], Maurice, M. Iwu1[1,5]
[1]International Centre for Ethnomedicine and Drug Development, 110 Aku Road, Nsukka Nigeria;
[2]Chemistry Department, University of Dschang, Box 67, Dschang, Cameroon.
[3]Berhanu M. Abegaz, Department of Chemistry, Faculty of Science, University of Botswana, P.O.Box UB0074, Gaborone, Botwana.
[4]Haskins Laboratories and Dept. of Biology, Pace University, New York, NY 10038-1502.
[5]Bioresources Development & Conservation Programme,11303 Amherst Ave # 2, Silver Spring, MD 2090

Abstract:

As part of ongoing research, under the auspices of International Cooperative Biodiversity Groups (ICBG), we have collected and identified plant materials used in West and Central African ethnomedicine in the treatment of infectious diseases including, African sleeping sickness, fevers and resistant malaria. Our *in vitro* antiprotozoal assays indicate that members of the family Fabaceae were among those that significantly inhibited the growth of *Plasmodium falciparum* and *Trypanosoma* spp. Out of 35 extracts from 17 species, eighteen extracts were active against CQ-sensitive (D6) while 17 were active against CQ-resistant (W2) strains of *Plasmodium falciparum with* IC_{50} < or = 50 μg/mL. The most active extracts on both strains were those of Millettia *griffoniana*, *Glycyrrhiza lepidota*, *Anthonotha crassifolia, Albizia ferruginea* and *Cassia fasciculata* with IC_{50} < or = 5μg/mL. Significant antitrypansomal activity was found for *Erythrina senegalensis* against *Trypanosoma b. brucei*, EATRO 110, *Trypanosoma rhodesiense* KETRI 243, *T. rhodesiense* 243 As 10-3 strains with corresponding IC_{50} values of 7.2, 9.1 and 14.75μg/mL. Bioassay directed-fractionation of the extracts from the seeds of *Millettia griffoniana,* often cited by traditional medicine practitioners yielded two new isoflavonoid compounds (7-ethoxyebenosin, and griffonianone F) and two known (7-methoxy-8, (3,3-diethylallyl)isoflavone) and Calopogonium isoflavone),which demonstrated strong antiplasmodial and antitrypasomal activities.

Trypanocidal Diarylheptanoids from *Aframomum letestuianum*

Pierre Kamnaing,[†] Apollinaire Tsopmo,[‡] Eric A. Tanifum,[†] Marguerite H. K. Tchuendem,[†] Pierre Tane,[†] Johnson F. Ayafor,[†,∥] Olov Sterner,*[,‡] Donna Rattendi,[§] Maurice M. Iwu,[⊥] Brian Schuster,[⊥] and Cyrus Bacchi*[,§]

Department of Chemistry, University of Dschang, Box 67, Dschang, Cameroon, Department of Organic and Bioorganic Chemistry, Lund University, P.O. Box 124, SE 221 00 Lund, Sweden, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100, and Haskins Laboratories and Department of Biology, Pace University, New York, New York 10038-1502

Received August 23, 2002

Three new diarylheptanoids, (4*Z*,6*E*)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)hepta-4,6-dien-3-one, letestuianin A (**1**), (4*Z*,6*E*)-5-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-4,6-dien-3-one, letestuianin B (**2**), and 1,7-bis(4-hydroxyphenyl)heptan-3,5-dione, letestuianin C (**3**), as well as the known (4*Z*,6*E*)-5-hydroxy-1,7-bis(4-hydroxyphenyl)hepta-4,6-dien-3-one (**5**) were isolated from *Aframomum letestuianum*. The known flavonoids 3-acetoxy-5,7,4′-trihydroxyflavanone, 3-acetoxy-7-methoxy-5,4′-dihydroxyflavanone, 7-methoxy-3,5,4′-trihydroxyflavone, and 3,3′,4′,5,7-pentahydroxyflavan were also obtained from this plant. Their structures were determined using a combination of 1D and 2D NMR techniques. The four diarylheptanoids were tested for growth inhibitory activity in vitro versus bloodstream forms of African trypanosomes. IC_{50} values in the range of 1−3 μg/mL were found for compounds **3** and **5**.

The genus *Aframomum* K. Schum belongs to the economically and medicinally important family Zingiberaceae. It is represented in Cameroon by over 20 species of rhizomatous herbs.[1] All of them are widely used locally in ethnodietary and in folk medicinal preparations as well as for cultural and spiritual purposes.[2] In our previous research on this genus, we reported the isolation and characterization of several flavonoids and labdane diterpenes.[3−5] In continuation of our work on this genus and as part of our efforts to discover new antiparasitic drug leads from Cameroonian medicinal plants[6] we have investigated the seeds of *Aframomum letestuianum* and herein report the isolation of four diarylheptanoids. Three are new compounds to which we have given the trivial names letestuianin A (**1**), letestuianin B (**2**), and letestuianin C (**3**). The fourth is the previously reported (4*Z*,6*E*)-5-hydroxy-1,7-bis(4-hydroxyphenyl)hepta-4,6-dien-3-one (**5**).[7] In addition, the known flavonoids 3-acetoxy-5,7,4′-trihydroxyflavanone,[4] 3-acetoxy-7-methoxy-5,4′-dihydroxyflavanone,[4] 7-methoxy-3,5,4′-trihydroxyflavone,[5] and 3,3′,4′,5,7-pentahydroxyflavan[8] were isolated in large quantitities. The trypanocidal activity of the diarylheptanoids is presented.

Results and Discussion

A sample of the air-dried powdered seeds of *A. lestestuianum* was extracted with MeOH−CH_2Cl_2 and subjected to sequential extraction with hexane and CH_2Cl_2. Bioassay-guided fractionation and purification of the CH_2Cl_2-soluble fractions led to the isolation of four diarylheptanoids and four flavonoids. The structures of the compounds were elucidated by spectroscopic techniques, and comparison with literature data revealed that three of the isolated diarylheptanoids are new compounds.

Compound **1** was obtained as a yellowish oil. The EIMS spectrum showed a molecular ion peak at *m/z* 340 with 100% intensity, compatible with the molecular formula $C_{20}H_{20}O_5$. The IR spectrum showed important absorption bands at ν_{max} 3363 (OH) and 1633 cm^{-1} (C=C−C=O). The ^1H NMR spectrum revealed the presence of a *para*-disubstituded benzene ring characterized by signals at δ 7.52 (2H, d, J = 8.5 Hz) and 6.88 (2H, d, J = 8.5 Hz); a 1,3,4-trisubstituted benzene ring [δ 6.85 (H-2″, d, J = 2.0 Hz), 6.72 (H-5″, d, J = 8.4 Hz), and 6.68 (H-6″, dd, J = 8.4, 2.0 Hz)]; a pair of *trans* olefinic protons at δ 7.53 (H-7, d, J = 15.9 Hz) and 6.53 (H-6, d, J = 15.9 Hz); a methoxy signal at δ 3.80 (s); and two methylenes at δ 2.85 and 2.67 (each triplet, J = 8.1 Hz). This was in sound agreement with the ^{13}C NMR spectrum (Table 1), which showed signals attributed to a carbonyl at δ 199.9 (C-3) and a hydroxylated olefinic carbon at δ 178.5, which with subsequent HMBC cross correlation peak with the *trans* olefinic protons as well as with H-4 (δ 5.81) was attributed to C-5. Three oxygenated sp^2 carbon atoms were also observed at δ 145.9, 148.3, and 160.5. A judicious analysis of the $^1H-^1H$ COSY data of **1** implied connectivities of H-7 to H-6, H-2 to H-1, H-2′ to H-3′ and H-6′, H-5′ to H-3′ and H-6′, and H-6″ to H-2″ and H-5″. The correlations observed in the NOESY and HMBC spectra attached the methoxy group at position C-3″ rather than C-4″, and pertinent correlation peaks were observed between the OMe group (δ 3.80) and H-2″ (δ 6.85) in the NOESY spectrum and between the OMe protons and C-3″ in the HMBC spectrum. The stereochemistry of the C-6/C-7 double bond bond is *E* as judged by the coupling constant between the two protons (J = 15.9 Hz), and that of the C-4 double bond is *Z*, as a clear NOESY correlation peak was observed between H-4 and H-6. Further analysis of HMBC and NOESY spectra led to the assignment of all carbons and protons, and the structure of compound **1** is (4*Z*,6*E*)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)hepta-4,6-dien-3-one. The trivial name letestuianin A was given to this new diarylheptanoid.

Compound **2** was obtained as yellow needles (CH_2Cl_2), mp 179−180 °C. The EIMS of **2** showed a molecular ion peak at *m/z* 370 compatible with the molecular formula $C_{21}H_{22}O_6$. The IR spectrum showed absorption bands due

* To whom correspondence should be addressed. Tel: (46) 46 222 8213. Fax: (46) 222 8209. E-mail: Olov.Sterner@bioorganic.lth.se (O.S). Tel: (1) 212 346 1246. Fax: (1) 212 346 1586. E-mail: cbacchi@fsmail.pace.edu (C.B).
† University of Dschang.
∥ Deceased.
‡ Lund University.
§ Pace University.
⊥ Walter Reed Army Institute of Research.

10.1021/np020362f CCC: $25.00 © 2003 American Chemical Society and American Society of Pharmacognosy
Published on Web 02/19/2003

Table 1. ^{13}C (125 MHz) and ^{1}H NMR (500 MHz) Data for Compounds **1**, **2**, and **3**

	1[a]		**2**[b]		**3**[c] (major component)	
position	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	31.7	2.85 t (8.1)	30.4	2.77 t (8.0)	29.7	2.71 s
2	42.7	2.67 t (8.1)	41.3	2.65 t (8.0)	46.4	2.71 s
3	199.5		199.2		206.5	
4	101.0	5.81 s	100.2	5.90 s	57.4	3.52 s
5	178.5		177.9		206.5	
6	120.8	6.53 d (15.9)	119.7	6.63 d (15.9)	46.4	2.71 s
7	140.6	7.53 d (15.9)	140.2	7.45 d (15.9)	29.7	2.71 s
1′	127.8		126.3		133.0	
2′	130.9	7.52 d (8.5)	111.0	7.25 d (2.0)	130.4	6.98 d (8.5)
3′	116.9	6.88 d (8.5)	148.0		116.3	6.68 d (8.5)
4′	160.5		149.2		156.7	
5′	116.9	6.88 d (8.5)	115.7	6.78 d (8.0)	116.3	6.68 d (8.5)
6′	130.9	7.52 d (8.5)	123.0	7.13 dd (8.0, 2.0)	130.4	6.98 d (8.5)
1″	133.4		131.6		133.0	
2″	112.9	6.85 d (2.0)	112.5	6.77 d (2.0)	130.4	6.98 d (8.5)
3″	148.3		147.4		116.3	6.68 d (8.5)
4″	145.9		144.7		156.7	
5″	115.8	6.72 d (8.4)	115.3	6.64 d (7.9)	116.3	6.68 d (8.5)
6″	121.6	6.68 dd (8.4, 2.0)	120.3	6.59 dd (7.9, 2.0)	130.4	6.98 d (8.5)
OMe′			55.7	3.78 s		
OMe″	56.3	3.80 s	55.5	3.71 s		

[a] Spectra recorded in acetone-d_6. [b] Spectra recorded in DMSO-d_6. [c] Spectra recorded in CD_3OD.

to hydroxyl group(s), enone, and aromatic ring(s) functionalities at ν_{max} 3436, 1631, and 1602 cm^{-1}, respectively. The ^{1}H and ^{13}C NMR data (Table 1) of **2** were closely related to those of compound **1**. The only significant differences compared to **1** are that both aromatic systems are 1,3,4-trisubstituted and the presence of an additional methoxy group in **2**. Once more the NOESY spectrum was useful for the determination of the position of the methoxy groups on the aromatic rings as well as for the *Z* conformation of one of the double bond. Important correlation peaks were observed between the OMe at δ 3.71 and the proton at δ 6.77 (d, $J = 2.0$ Hz) as well as the OMe at δ 3.78 and the proton at δ 7.25 (d, $J = 2.0$ Hz). Together with COSY and HMBC data the structure (4*Z*,6*E*)-5-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-4,6-dien-3-one was determined for compound **2**, and it was given the trivial name letestuianin B.

Compound **3** was obtained as a pale yellow oil. The EIMS spectrum of **3** showed a molecular ion peak at *m/z* 312 compatible with the molecular formula $C_{19}H_{20}O_4$. The IR spectrum showed absorption bands at ν_{max} 3407, 1630, 1613, 1515, and 828 cm^{-1} closely related to those of **1** and **2**. The 1D NMR spectra suggested the presence of two components, in a 3:7 ratio. For the major component, the ^{1}H NMR spectrum indicated the presence of a *para*-disubstituted benzene ring [δ 6.98 (2H, d, $J = 8.5$ Hz) and 6.68 (2H, d, $J = 8.5$ Hz)] and two methylenes appearing as singlet at δ 2.71. An isolated proton appeared at δ 3.52 as a singlet. The intensity of the latter signal was very low due to exchange with deuterium from the methanol solvent used for NMR experiments. These data account only for nine protons, and the fact that only eight carbon signals appeared in its ^{13}C NMR spectrum suggests that **3** is symmetric with two identical benzene rings. The data for the major component were compatible only with the 1,3-diketone shown in Figure 1, and as expected, this is in equilibrium with an enol tautomer. Typical signals for the enol appeared in the ^{1}H and ^{13}C NMR spectra, for example a proton signal at δ 4.58 (H-4) and carbon signals at δ 194.7 (C-3) and 100.0 (C-4), but to confirm this tautomeric equilibrium, compound **3** was treated with a mixture of pyridine–Ac_2O (1:1) to give the acetylated derivative **4**. The analysis of the ^{1}H NMR spectrum of **4** revealed the presence of a 1,4-disubstituted benzene ring, showing that

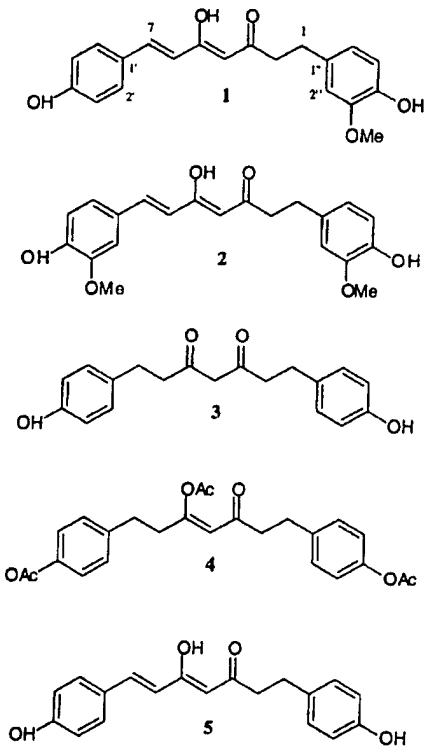

Figure 1.

the symmetric nature of the molecule had been distorted. An olefinic signal was also observed in **4** at δ 5.42 in replacement of the methylene signal that was present at δ 3.52 in **3**. The presence of three acetate functions was characterized by shifts at δ 2.22 (6H, s) and 2.10 (3H, s). The analysis of the ^{13}C NMR spectrum of **4** with signals at δ 169.5, 169.7, and 170.0 confirmed the three acetate functions. A conjugated carbonyl function was also observed at δ 193.2. All the above information showed that **4** was the enol form of **3**. Further analysis of HMBC, COSY, and NOESY spectra of the nonacetylated and acetylated derivative led to the characterization of compound **3** as 1,7-bis(4-hydroxyphenyl)heptan-3,5-dione, consequently named letestuianin C.

Table 2. Antitrypanocidal Activities of *Aframomum letestuianum* Diarylheptanoids

compound	IC_{50} (μg/mL) Lab110 EATRO *T. b. brucei*	KETRI *T. b. rhodesiense* KETRI isolates 243	243 As 10−3
1	>100		
2	67	>100	>100
3	1.4	2.3	2.6
5	2.6	2.8	1.3
melarsoprol	0.002	0.0005	0.005
pentamidine	0.0006	0.0005	0.004

Previous studies on the genus *Aframomum* have, up to date, reported the presence of only two major classes of natural products, diterpenoids and flavonoids. To the best of our knowledge, **1**, **2**, **3**, and **5** are the first diarylheptanoids reported from this important genus, although they are common in the sister genera *Alpinia*[9−11] and *Curcuma*.[12−14] The four diarylheptanoids obtained were assayed for trypanocidal activity, tested against bloodstream forms of *Trypanosoma b. brucei* and *Trypanosoma b. rhodesiense* isolates grown in vitro in 24-well plates. Coulter counts were made daily, and the IC_{50} values determined after 48 h are given in Table 2. Compound **1** was not growth inhibitory below 100 μg/mL. Compound **2** gave an IC_{50} value of 67 μg/mL with the *T. b. brucei* isolate but >100 μg/mL with *T. b. rhodesiense* isolates. Compounds **3** and **5**, however, were highly effective in the range 1−3 μg/mL for all isolates tested. Interestingly, the additional methoxy group in **1**, compared to **5**, makes it inactive. Corresponding IC_{50} values for the trypanocides melarsoprol and pentamidine were ~300−5000-fold lower; however, lack of sufficient material prevented us from testing these compounds in vivo in a mouse model infection.

Experimental Section

General Experimental Procedures. Melting points were recorded with a Reichter microscope and are uncorrected. The UV and IR spectra (KBr) were recorded with a Shimadzu UV-3001 and a Jasco FT-IR spectrophotometer, respectively. ^1H NMR and ^{13}C NMR were recorded in $CDCl_3$, acetone-d_6, DMSO-d_6, or CD_3OD using a Bruker ARX500 spectrometer with an inverse multinuclear 5 mm probe head equipped with a shielded gradient coil. The chemical shifts (δ) are reported in parts per million relative to tetramethylsilane (TMS, δ = 0), while the coupling constants (*J*) are given in Hz. COSY, HMQC, and HMBC experiments were recorded with gradient enhancements using sine-shaped gradient pulses. For 2D heteronuclear correlation spectroscopy the refocusing delays were optimized for $^1J_{CH}$ = 145 Hz and $^nJ_{CH}$ = 10 Hz. The raw data were transformed and the spectra evaluated with the standard Bruker UXNMR software. The positive EI (70 eV) and CI mass spectra were recorded with a JEOL SX102 spectrometer. Column chromatography was run on Merck Si gel 60 and gel permeation on Sephadex LH-20. TLC analyses were carried out on Si gel GF_{254} precoated plates with detection accomplished by spraying with 50% H_2SO_4 followed by heating at 100 °C, or by visualizing with a UV lamp at 254 and 366 nm.

Plant Material. The seeds of *A. letestuianum* were collected from Abong-bang, East Province, Cameroon, in December 1998. Mr. Paul Mezili, a retired botanist of the Cameroon Herbarium, authenticated the plant material. Voucher specimens (BUD 0391) were deposited at the Herbarium of the Botany Department of the University of Dschang.

Extraction and Isolation. The air-dried powdered seeds of *A. letestuianum* (2 kg) were macerated with a mixture (1:1) of MeOH−CH_2Cl_2 (4 L) overnight and evaporated in vacuo to yield a crude extract (150.5 g). This crude extract was dissolved in 80% MeOH (600 mL) and extracted hexane (3 × 500 mL). The aqueous MeOH was further diluted with water to 60%MeOH and extracted with CH_2Cl_2 (3 × 500 mL). Vacuum concentration yielded CH_2Cl_2 extract (36.5 g) and hexane extract (28.0 g), which contained mostly fats. Subjection of the CH_2Cl_2 extract to column chromatography over silica gel eluting with a CH_2Cl_2−hexane gradient followed by acetone−CH_2Cl_2 afforded three major fractions, I [500 mg, CH_2Cl_2−hexane (6:4)], II [16.0 g, CH_2Cl_2−hexane (8:2) and acetone−CH_2Cl_2 (1:9)], and III [2.1 g, acetone−CH_2CL_2 (2:8)]. Subjecting fraction I to repeated column chromatography on silica gel eluted with a CH_2Cl_2−hexane gradient and further purification by gel permeation chromatography on Sephadex LH-20 (MeOH) afforded compounds **1** (24 mg), **2** (10.4 mg), and 7-methoxy-3,5,4′-trihydroxyflavanone (5.5 mg). Subjection of fraction II (7.5 g) to gel permeation chromatography on Sephadex LH-20 (MeOH) gave additional amount of **1** (15 mg), 3-acetoxy-5,7,4′-trihydroxyflavanone (3.5 g), 3-acetoxy-7-methoxy-5,4′-dihydroxyflavanone (1.8 g), and a mixture of two main products (350 mg), which was further purified by countercurrent chromatography (CCC) eluting head to tail with hexane−ethyl acetate−MeOH−H_2O (4:6:5:5) and reversing the flow after 3 h to obtain compounds **3** (179 mg) and **5** (86 mg). Treatment of fraction III on a silica gel column eluted with MeOH−CH_2Cl_2 gradient followed by gel permeation on Sephadex LH-20 (MeOH−CH_2Cl_2, 1:1) afforded 3,3′,4′,5,7-pentahydroxyflavan (139 mg) and a mixture of nonresolved compounds.

(4*Z*,6*E*)-5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)hepta-4,6-dien-3-one, letestuianin A (1): yellowish oil; UV (MeOH) λ_{max} (log ε) 380 (3.2) and 283 (3.9) nm; IR (KBr) ν_{max} 3363, 2937, 1633, 1583, 1514, 1431, 831, and 790 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* 340 $[M]^+$ (100), 322 (10), 189 (30), 147 (70), 137 (55), 107 (18); HREIMS *m/z* 340.1304 (calcd for $C_{20}H_{20}O_5$, 340.1311).

(4*Z*,6*E*)-5-Hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-hepta-4,6-dien-3-one, letestuianin B (2): shiny yellow needles (CH_2Cl_2−hexane); mp 179−180 °C; UV (MeOH) λ_{max} (log ε) 374 (2.9) and 288 (3.4) nm; IR (KBr) ν_{max} 3436, 1631, 1602, 1511, 1280, 1202, 1028, and 814 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* 370 $[M]^+$ (44), 352 (16), 219 (18), 177 (63), 137 (100), 44 (25); HREIMS *m/z* 370.1411 (calcd for $C_{21}H_{22}O_6$, 370.1416).

1,7-Bis(4-hydroxyphenyl)heptan-3,5-dione, letestuianin C (3): yellowish oil; UV (MeOH) λ_{max} (log ε) 279 (3.4) and 224 (2.4) nm; IR (KBr) ν_{max} 3407, 1623, 1613, 1515, 1462, 1385, 1243, 828 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* 312 $[M]^+$ (34), 191 (10), 120 (20), 107 (100), 77 (10); HREIMS *m/z* 312.1358 (calcd for $C_{19}H_{20}O_4$, 312.1361).

Acetylation of Letestuianin C (3). Compound **3** (25 mg) was dissolved in a (1:1) mixture of pyridine−Ac_2O (4 mL) and the reaction mixture left at room temperature overnight. The product was concentrated with addition of toluene and purified on a silica gel column (hexane−EtOAc, 9:1) to give 5-acetoxy-1,7-bis(4-acetoxyphenyl)hepta-4-en-3-one (**4**) (26 mg) as a colorless oil: ^1H NMR ($CDCl_3$, 500 MHz) δ 2.10 (Ac), 2.22 (2 × Ac), 2.52 (4H, t, *J* = 7.6 Hz, H-2, H-6), 2.81 (4H, t, *J* = 7.6 Hz, H-1, H-7), 5.42 (H-4, s), 6.90 (4H, m, H-2′, H-6′, H-2″, H-6″), 7.23 (4H, m, H-3′, H-5′, H-3″, H-5″); ^{13}C NMR $CDCl_3$, 125 MHz) δ 31.2 (C-1, C-7), 40.3 (C-2, C-6), 100.1 (C-4), 121.9 (C-3″, C-5″), 122.0 (C-3′, C-5′) 129.7 (C-2″, C-6″), 129.8 (C-2′, C-6′), 138.5 (C-1″), 138.6 (C-1′), 149.4 (C-4′, C-4″), 179.1 (C-5), 193.2 (C-3).

Biological Assay. Assays for inhibition of trypanosomal growth were conducted as previously described.[15,16] Bloodstream-form trypanosomes were cultured in modified IMDM with 20% horse serum at 37 °C. Drug studies were done in duplicate in 24-well plates (1 mL/well) with final inhibitor concentrations of 0.1, 1, 10, 25, and 100 μg/mL. Wells were inoculated with 10^5 trypanosomes, and one-half the volume of each well was changed daily. After 48 h, the parasite number was determined in a Model Z1 Coulter counter and IC_{50} values were calculated from semi-log plots. Assays were done two or more times, using widely spaced concentration curves initially, followed by curves of closely spaced values to obtain the IC_{50} value.

Compounds were dissolved in 100% dimethyl sulfoxide and diluted in medium, so that the dimethyl sulfoxide concentration never exceeded 0.3%, a noninhibitory concentration.

Strains used were *Trypanosoma b. brucei* Lab 110 EATRO and Kenya Trypanosomiasis Research Institute (KETRI) isolates *Trypanosoma b. rhodesiense* 243 and 243 As 10-3.[15,16]

Acknowledgment. The authors gratefully acknowledge financial support from the Fogarty International Center (NIH Grant 1U01TWO 0317, as part of the International Cooperative Biodiversity Group Program) and the Swedish Natural Science Research Council.

References and Notes

(1) Koechlin, J. F. *Flore du Cameroun. Scitaminales*; Muséum National de l'Histoire Naturelle: Paris, 1965; p 4.
(2) Thomas, D. W.; Thomas, J.; Bromley, W. N.; Mbenkum, F. T. *Korup Ethnobotany Survey, Final Report to: The World Wide Fund for Nature*; Penda House: Weyside Park, Godalming: Surrey, U.K.; 1989.
(3) Ayafor, J. F.; Tchuendem, M. K. H.; Nyasse, B.; Tillequin, F.; Anke, H. *Pure Appl. Chem.* **1994**, *66*, 2327−2330.
(4) Ayafor, J. F.; Connolly, J. D. *J. Chem. Soc., Perkin Trans. 1* **1981**, 2563−2565.
(5) Tsopmo, A.; Tcheundem, M. K. H.; Ayafor, J. F.; Tillequin, F.; Kock, M.; Anke, H. *Nat. Prod. Lett.* **1996**, *9*, 93.
(6) Tchuendem, M. H. K.; Mbah, J. A.; Tsopmo, A.; Ayafor, J. F.; Okunji, C.; Iwu, M. M.; Schuster, B. M. *Phytochemistry* **1999**, *52*, 1095−1099.
(7) Hui, D.; Sao-Xing, C. *J. Nat. Prod.* **1998**, *61*, 142−144.
(8) Aldrich Libray of ^{13}C and ^{1}H FT NMR spectra **1992**, *2*, 326A.
(9) Kadota, S.; Hui, D.; Basnet, P.; Prasain, J. K.; Xu, G.; Namba, T. *Chem. Pharm. Bull.* **1994**, *42*, 2647−2649.
(10) Itokawa, H.; Morita, M.; Midorikawa, I.; Aiyama, R.; Morita, M. *Chem. Pharm. Bull.* **1985**, *33*, 4889−4893.
(11) Kiuchi, F.; Shibuya, M.; Sankawa, U. *Chem. Pharm. Bull.* **1982**, *30*, 2279−2282.
(12) Kiuchi, F.; Goto, Y.; Sugimoto, N.; Akao, N.; Kando, K.; Tsuda, Y. *Chem. Pharm. Bull.* **1992**, *41*, 1640−1643.
(13) Masuda, T.; Jitoe, A.; Nakatani, N.; Yonemori, S. *Phytochemistry* **1993**, *33*, 1557−1560.
(14) Uehara, S.; Yasuda, I.; Akiyama, K.; Morita, H.; Takeya, K.; Itokawa, H. *Chem. Pharm. Bull.* **1978**, *35*, 3298−3304.
(15) Hirumi, H.; Hirumi, L. *J. Parasitol.* **1989**, *75*, 985−989.
(16) Sufrin, J. R.; Rattendi, D.; Spiess, A. J.; Lane, S.; Marasco, C. J., Jr.; Bacchi, C. J. *Antimicrob. Agents Chemother.* **1996**, *40*, 2567−2572.

NP020362F