Antimalarial Natural Products from some Cameroonian Medicinal Plants

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Abstract Natural products drug discovery has proven productive in the identification of lead antimalarial substances like (quinine and artemisinin). Continuing with the inventory and collection of plants used traditionally to treat malaria, the extracts of three plant species, *Araliopsis tabuensis*, *Odyendyea gabunensis* and *Penianthus longifolius* were found to be the most active. They were evaluated in the antimalarial screen applying continuous in vitro cultures of asexual erythrocyte stages of two *Plasmodium falciparum* strains (D-6 and W-2). Antiplasmodial-guided fractionation of the extracts using various chromatographic methods afforded respectively 13 alkaloids from the stem bark of *A. tabuensis*, 3 alkaloids and one quassinoid from the stem bark of *O. gabunensis*, and finally 3 alkaloids from *P. longifolius*. The structures of these compounds were elucidated using modern spectroscopic techniques. Many of these compounds display noteworthy antiplasmodial activity, compared to the commercial antimalarial drugs, chloroquine and mefloquine. The quassinoid, alantinone, the most active, could be a good candidate for antimalarial drug development.

Résumé La découverte des médicaments à partir des substances naturelles a contribué à l'identification de drogues antipaludéennes (quinine, artémisinine). Continuant avec l'inventaire et la collecte des plantes utilisées en médecine traditionnelle pour soigner le paludisme, les extraits de trois plantes, *Araliopsis tabuensis*, *Odyendyea gabunensis* et *Penianthus longifolius* se sont montrés les plus actifs. Ils ont été évalués par un test antipaludéen contre deux souches (D-6 et W-2) du *Plasmodium falciparum*. Le fractionnement bio-guidé de ces extraits à l'aide de diverses méthodes chromatographiques a fourni respectivement 13 alcaloïdes des écorces du tronc de *A. tabuensis*, 3 alcaloïdes et un quassinoïde des écorces du tronc de *O. gabunensis*, et finalement 3 alcaloïdes de *P. longifolius*. Les structures de ces composés ont été élucidées à l'aide des techniques spectroscopiques modernes. Certains de ces métabolites isolés montrent une activité antipaludéenne significative, comparés aux antipaludéens commerciaux, chloroquine et méfloquine. Le quassinoïde, alantinone, composé le plus actif, est un potentiel candidat pour le développement des médicaments antipaludéens.

Introduction

Malaria remains the greatest human killer among the parasitic diseases, despite the world-wide effort to combat the disease and attempts to eradicate the causative organism. The emergence of multi-drug resistant strains of *Plasmodium falciparum*, the most dangerous of the malaria parasites, poses a serious health care problem, not only in malaria-endemic countries but also among international travellers. It is therefore imperative to develop more effective and orally active anti-malarials.

Tropical plants have offered and still offer a viable source for anti-malarial drugs. Quinine, isolated from the bark *Cinchona* species, was the first anti-malarial (Beckmann et al., 1998). Chloroquine and other synthetic quinoline-containing anti-malarial drugs, including other 4-aminoquinolines and the recent mefloquine, all owe their development from the parent drug, quinine. More recently discovered was the anti-malarial artemisinin from the Chinese medicinal plant *Artemisia annua* (Klayman et al., 1985) Based on the history of anti-malarial drugs, natural products drug discovery has proven productive both in the use of the extracts and in the identification of lead structures for synthetic derivatives. The search for lead compounds for anti-malarial development is a subject of much current research, involving both plants used in traditional medicine and those considered to be a potential source of novel chemical structures. However, several studies have demonstrated that there is significantly higher rate of bioactivity in plants used in traditional medicine compared to those randomly collected. For the past few years, one of the main objectives of our research project has been the inventory and collection of plants used traditionally to treat malaria. A total of 112 species have been selected and after preliminary biotests, extracts of 17 of them displayed significant activity (table1) of which *Araliopsis tabuensis*, *Odyendyea gabunensis* and *Penianthus longifolius* were the most active.

Table1 Active anti malarial extracts

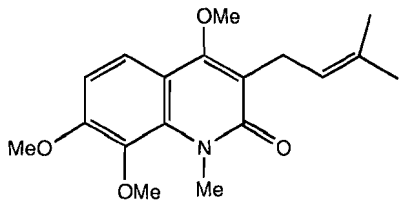
Name of the plant	IC_{50} ng / ml	
	D- 6	***W- 2***
1. *Neoboutonia velutina* $CHCl_3$	3679.	3797
2. *Cleiptopholis patens MeOH*	3597	> 5000
3. *Uvariodendron spp.*	71151	7894
4. *Xymalox monosperma*	1847	3399
5. *Pentadiplandra brazzeana*	4191	6898
6. *Microglossa afzelli*	4808	3333
7. *Araliopsis tabouensis*	895.65	1042
8. *Odyendyea gabonensis*	111.99	101.54
9. *Phyllantus muellerianus*	3787	366.67
10. *Glossocalyx brevipes*	1972	978.10
11. *Reneilmia cincinnata*	4136	2551
12. *Millettia griffoniana*	3868	2829
13. *Khaya anthotheca*	> 5000	1468
14. *Penianthus longifolius*	350.06	284.37
15. *Euphorbia poinsonni*	2968	1542
16. *Zanthoxylum lemarei*	2949	1768
17. *Pycnanthus angolensis*	530.33	1800

Growth inhibition assay

The *in vitro* anti-plasmodial assays were determined at the Walter Reed Army Institute of Research using a modification of semi-automated microdilution technique described earlier (Desjardins et al., 1979). Two *Plasmodium falciparum* malarial parasite clones designated Indochina (W-2) and Sierra Leone (D-6) were utilised in susceptibility tests. The W-2 clone is susceptible to mefloquine but resistant to chloroquine, sulfadoxine, pyrimethamine, and quinine, whereas the D-6 clone is naturally resistant to mefloquine but susceptible to chloroquine, sulfadoxine, pyrimethamine, and quinine. The tested compounds were dissolved in DMSO and serially diluted using malarial growth medium. The parasites were exposed to serial dilutions of each compound for 48 h and incubated at 37 °C with 5% O2, 5% CO2, and 90% N2 prior to the addition of [3H]hypoxanthine. After a further incubation of 18 h, parasite DNA was harvested from each microtiter well using Packard Filtermate 196 Harvester (Meriden, CT) onto glass filters. Uptake of [3H]hypoxanthine was measured with a Packard top count scintillation counter. Concentration response data were analyzed by a nonlinear regression logistic dose response model, and the IC_{50} values (50% inhibitory concentrations) for each compound were calculated Drug-induced reduction in uptake of tritiated hypoxantine was used as index of inhibition of parasite growth. Chloroquine diphosphate (Sigma, USA) was also used as control. The IC_{50} of chloroquine diphosphate was 0.003 μ g/ml for the D-6 strain and 0.079μ g/ml for the W-2 strain.

Araliopsis tabouensis

Araliopsis tabouensis Aubrev & Pellegr (Rutaceae) is a tall tree occurring in the humid West African forest (Letouzey, 1963; Irvine et al., 1961). In the course of the ethbotanical survey in the Bakundu tribe village of Toko, it was discovered that the stem bark of the plant *Araliopsis tabouensis* is used for the treatment of fevers, characteristic symptom of malaria. Infusion of the dried bark of this plant in palm-wine or «local gin («afo-fo ») is drunk against fevers. The air-dried powdered stem bark of *A. tabouensis* was macerated in CH_2Cl_2:MeOH (1:1) followed by partition to obtain *in vitro* bioactive methylene chloride extract (IC_{50} 2.23 and 1.22 μg/ml for D-6 and W-2 respectively) and methanol extract (IC_{50} 895.65 and 1042.1ng/ml for the D-6 and W-2 strains respectively). Bioassay-guided fractionation and purification of the two extracts led to isolation of 13 Alkaloids identified to N-methylpreskimmianine (**1**) (Ayafor et al., 1980), veprisine (**2**) (Ayafor et al., 1980), vepridimerine A (**3**) (Ngadjui et al., 1982; 1988), vepridemerine B (**4**) (Fish et al., 1972), vepridimerine C (**5**) (Ngadjui et al., 1982 ; 1988), araliopdimerine A (**6**) (Ngadjui et al., 1988), skimmianine (**7**) (Fish et al., 1972), kokusaginine (**8**) (Fish et al., 1972), Maculine (**9**) (Fish et al., 1972), flindersiamine (**10**) (Ngadjui et al., 1982), platydesmine (**11**) (Ngadjui et al., 1982), ribalinine (**12**) (Vaquette et al., 1976) and araliopsinine (**13**) (Ngadjui et al., 1988). For each compound isolated, melting point, optical rotation, UV, IR, proton NMR, carbon-13 NMR, and mass spectra were recorded in order to characterize the molecule. Compounds of novel structure required a combination of these techniques with two-dimension NMR experiments such as ^1H-^1H-COSY, DEPT, NOESY, selective INEPT, HMQC, HMBC, etc. In some cases chemical modifications were necessary.



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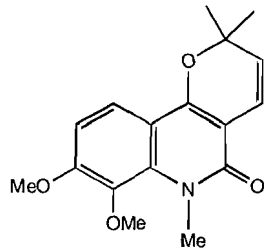

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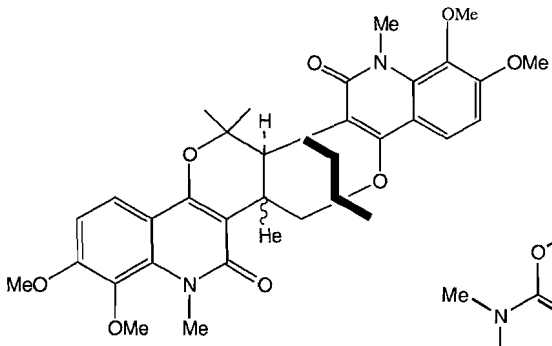
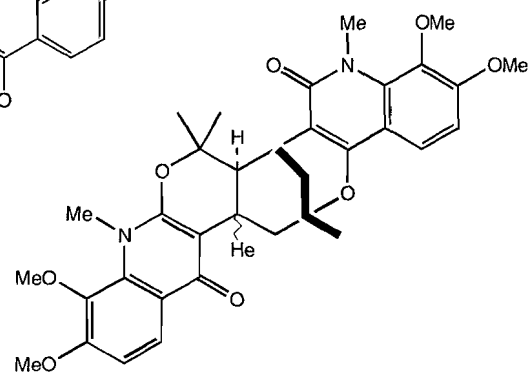


3 α-He
4 β-He



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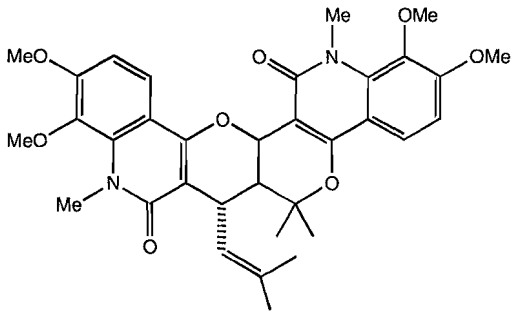

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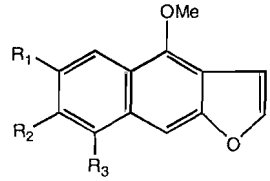
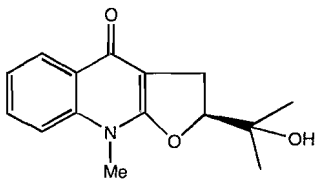


7 $R_2 = R_3 = OMe$, $R_1 = H$
8 $R_1 = R_2 = OMe$, $R_3 = H$
9 $R_1 + R_2 = O\text{-}CH_2\text{-}O$, $R_3 = H$
10 $R_1 + R_2 = O\text{-}CH_2\text{-}O$, $R_3 = OMe$



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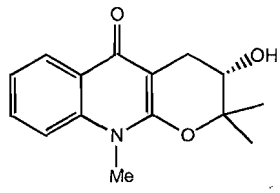

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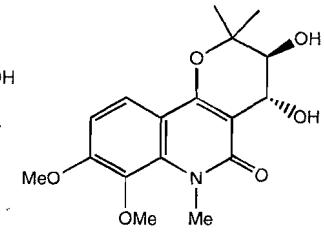

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The *in vitro* antiplasmodial activity of thirteen alkaloids against W-2 and D-6 clones of *Plasmodium falciparum* are shown in Table 2. The dimeric 2-quinolone alkaloid araliopdimerine-A (**6**) was the most active having IC_{50} values of 34 ng/mL and 170 ng/mL for the D-6 and W-2 strains respectively. Platydesmine (**11**) was also active, while N-methylpreskimmianine (**1**) and veprisine (**2**) showed only marginal activity. The rest of the compounds were inactive (IC_{50} >5000 ng/mL)

Table 2. *In vitro* Antimalarial Activity of *Araliopsis tabouensis* Alkaloids against W-2 and D-6 Clones of *Plasmodium falciparum*

Compounds	*Plasmodium falciparum* strains IC_{50} in ng/ml	
	Indochina (W-2)	Sierra Leone (D-6)
N-Methylpreskimmianine (**1**)	4690	1130
Veprisine (**2**)	>5000	1370
Vepridimerine A (**3**)	>5000	>5000
Vepridimerine B (**4**)	>5000	>5000
Vepridimerine C (**5**)		
Araliopdimerine-A (**6**)	170	34
Skimmianine (**7**)		
Kokusaginine (**8**)	>5000	>5000
Maculine (**9**)	>5000	>5000
Flindersiamine (**10**)	>5000	>5000
Platydesmine (**11**)	450	900
(-)-Ribalinine (**12**)		
Araliopsinine (**13**)	>5000	>5000
Chloroquine	79	3
Artemisinin	1	2
Mefloquine	1	9

From this table 2, it can be denoted that six alkaloids display good antiplasmodial activity (IC_{50} values =6µg/ml). Araliopdimerine-A, a dimeric quinolone alkaloid, showing the highest growth-inhibitory activity against the malarial parasite with IC_{50} values of 0.034 µg/ml and 0.176 µg/ml against D-6 and W-2, respectively followed by vepridimerine A.

Odyendyea gabonensis

Odyendyea gabonensis (Pierre) Engl. (Simaroubaceae) is a large rain forest tree encountered in Cameroon and Gabon (Aubreville, 1962). It is characterised by oval-oblong leaves with dark spots and with it attractive

flowers. The infusion of the stem bark is used by local population to cure malaria while the roots bark is used as antihelmentic (Forhas et al., 1982). The stem bark collected at Biba I, 15 Km of Ebolowa (South Cameroon) in September 2002 were extracted with the mixture CH_2Cl_2/MeOH (1/1) and the extract showed noteworthy activity for D-6 (IC_{50} 111.9917) and W-2 (IC_{50} 101.5394) plasmodial strains. Phytochemical examination of this extract led to isolation of three indole alkaloids identified to 11-hydroxycanthin-6-one (**14**) (Yishan et al., 1994), 4-hydroxycanthin-6-one (**15**) (Etherington et al., 1977), and 4-methoxycanthin-6-one (**16**) (Etherington et al., 1977), one quassinoid identified to ailanthinone (**17**) (Kuptan et al., 1975) and two tetracyclic triterpenoids, 3,21-dioxotirucalla-7,24-diene (**18**) (Mulholand et al., 1988) and 3-oxotirucalla-7,24-diene (**19**) (De Pascal et al., 1987). The pure compounds were subjected to *in vitro* antiplasmodial assays and the results summarised in table 3.

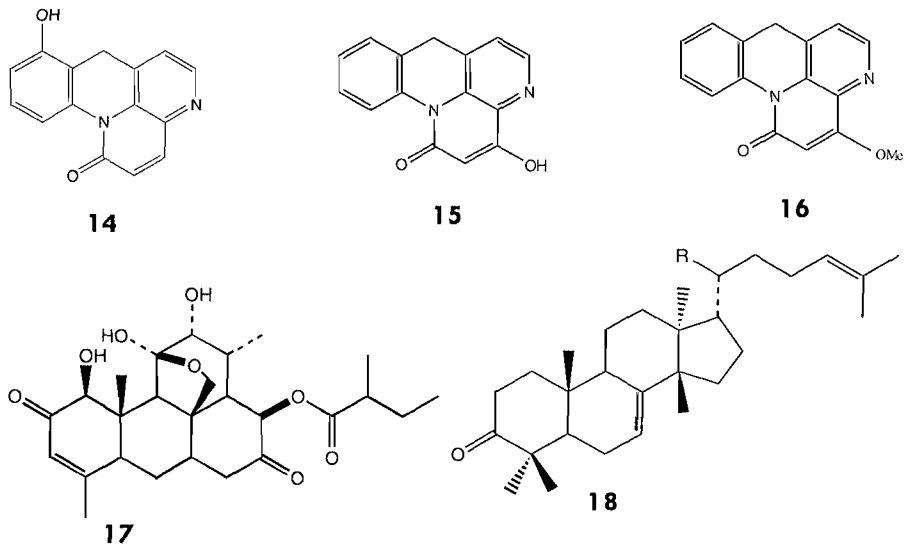


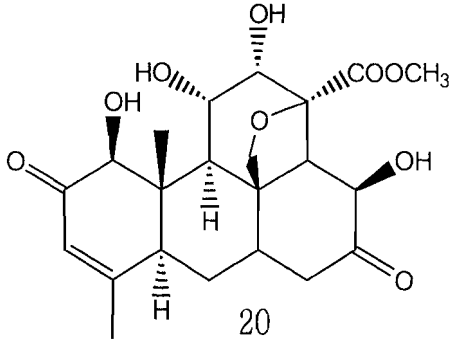
Table 3. *In vitro* Antimalarial Activity of *Odyendyea gabonensis* compounds against W-2 and D-6 Clones of *Plasmodium falciparum*

Compounds	*Plasmodium falciparum* strains IC_{50} in ng/ml	
	Indochina (W-2)	Sierra Leone (D-6)
11-hydroxycanthin-6-one (**14**)	118.1425	54.5319
4-hydroxycanthin-6-one (**15**)	>5000	>5000
4-methoxycanthin-6-one (**16**)	585.843	284.8271
Ailanthinone (**17**)	2.1548	2.5894
3,21-dioxotirucalla-7, 21-diene (**18**)	>5000	>5000
3-oxotirucalla-7, 21-diene (**19**)	>5000	>5000
Chloroquine	104.7298	4.6256
Mefloquine	2.0004	4.2499

The quassinoid ailanthinone was the most active having IC_{50} values of 2.5894 ng/ml and 2.1548 ng/ml for the D-6 and W-2 strains respectively while one of the alkaloid 11-hydroxycanthin-6-one shown moderated activities. The two other alkaloids (4-hydroxycanthin-6-one, and 4-methoxycanthin-6-one) and the two triterpenoids, 3,21-dioxotirucalla-7, 24-diene and 3-oxotirucIla-7, 24-diene were completely inactive. Comparing to the values obtained with the reference molecules IC_{50} of 4.6256 for chloroquine and 2.0004 for mefloquine, ailanthinone presents itself as a potential lead for anti-plasmodial drug development.

Structure Activity Relationships

Many quassinoids have been evaluated for their potential to inhibit the growth of plasmodia *in vitro* (Ilias et al., 2004). and the associated IC_{50} or EC_{50} values provide a natural structure activity relationship. From these data, a number of structural features appear crucial to anti-malarial potency of the quassinoids. These features are depicted in Figure 1. Most active anti-malarial quassinoids have a pentacyclic ring systems with a lactone D-ring and a methylene-oxygen bridge linking C-8 and C-11, as in ailanthinone, or linking C-8 and C-13, as in brusatol (**20**) (Saxena et al., 2003) When the methylene/oxygen bridge is absent, as in quassin (**21**), the anti-malarial activity is severely degraded. François et al (1998) who have prepared orally active chaparrinone derivatives, suggest that both the C-2-keto and the lack of C-14-hydroxy are important for both increased activity and decreased toxicity *in vivo*. Furthermore, most of the active quassinoids are also esterified at the C-15-hydroxyl, and the functionality of the ester R-group seems to be highly important. For example, ailanthinone differs from glaucarubinone (**22**) by only an additional hydroxyl group situated at C-2', yet the *in vitro* potency of the latter is 2.4 times greater and the *in vitro* toxicity is 2.5 times lower (Ilias et al., 2004). Ailanthinone could thus be used to prepare glaucarubinone-like derivatives through trans-esterification of the pendant ester. More recently, it has been discovered that C-3, C-15-bis-O-carbonate and bis-O-acetyl analogs of bruceolide are significantly less toxic *in vitro* and *in vivo*, and possess improved metabolic stability relative to natural bruceolide (**23**)(Wright et al., 1993). These data suggest that the anti-malarial activity and cytotoxicity are properties that are not directly related and can therefore be independently optimized.



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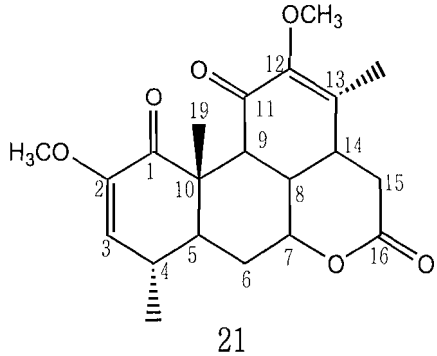

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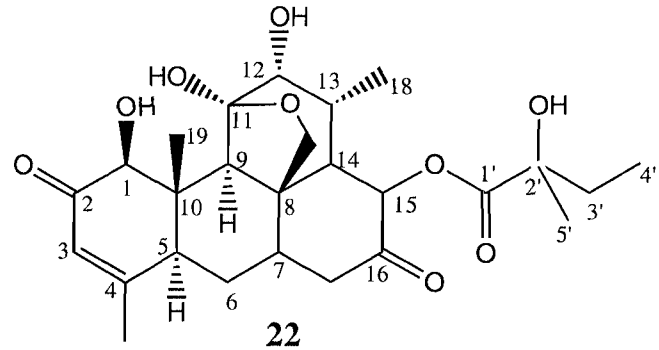

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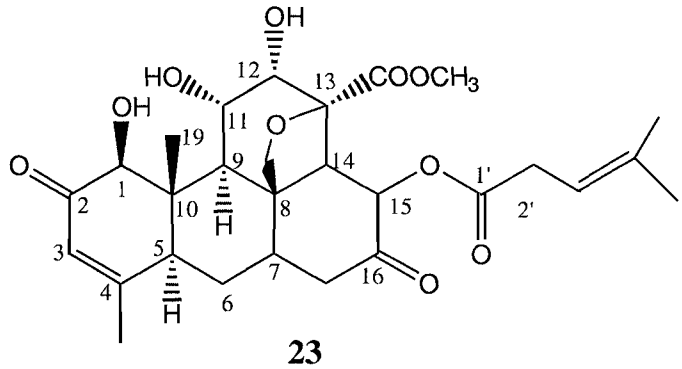

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Key features for anti-malarial activity

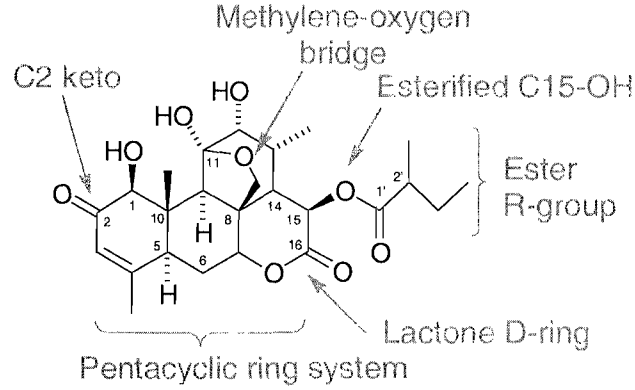


Figure 1: The key structural features of ailanthinone (17) that are believed to be

Addressing cytotoxic potential

Figure 2 identifies the structural features of ailanthinone that we believe are responsible, or partially responsible, for the observed toxicity of the natural product. Since these features appear to be relatively independent of those identified for anti-malarial activity (Figure1), we believe that modification of the appropriate functional groups will permit mitigation of the cytotoxic potential with concurrent optimization of the therapeutic index.

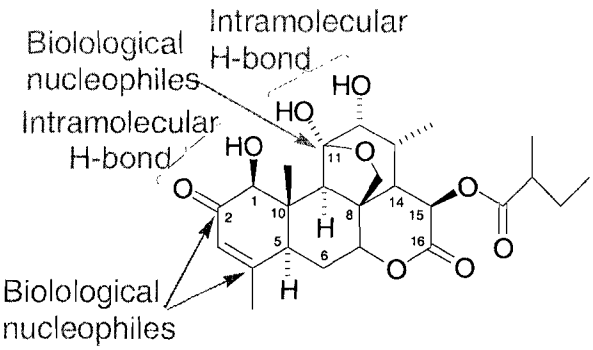
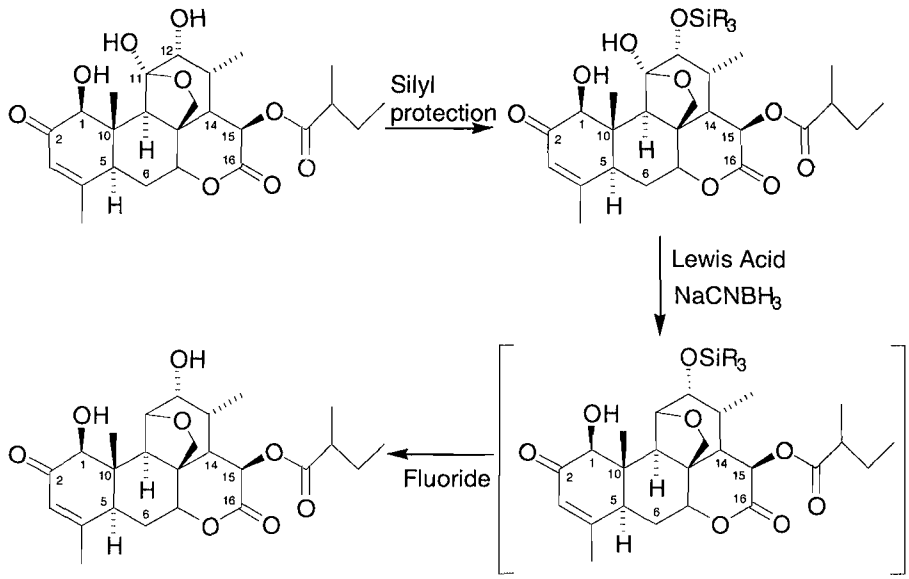


Figure 2: The structural features of ailanthinone that we believe may be responsible for the cytotoxicity associated with this and similar quassinoids.

A number of chemical transformations will be undertaken in order to test these hypothesized causes of toxicity and to explore ways in which we can address them. In both cases, keto/enol tautomerizations and intramolecular hydrogen bonding interactions are involved in the formation of the potentially reactive and toxic species. Disruption of the intramolecular hydrogen bonds through etherification, or deoxygenation at C1-OH and C12-OH can accomplish this. Alternatively, a potentially more stable, less toxic analog may be envisioned through conversion of the hemiketal to a cyclic ether via removal of the C10-OH. A possible synthetic route for this product is depicted in Scheme 1.

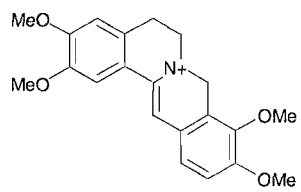


Scheme 1 Conversion of ailanthinone's hemiketal to a cyclic ether to eliminate the possible formation of the reactive oxonium species which may be a source of toxicity.

Penianthus longifolius

Penianthus longifolius Miers (Menispermaceae) is a small shrub visible as undergrowth of the forest (Murakami et al., 2003). The leaves are oval oblong, characterised by dark spots. The macerated stem bark is taken to manage fever and the roots are used as antihelminthic (André et al., 1995). The stem bark collected at mount Kala, near Yaounde in March 2001 was extracted with the mixture CH_2Cl_2/MeOH (1/1) and the extract showed good antiplasmodial activity against D-6 (IC_{50}=350.066 ng/ml) and W-2 (IC_{50}=284.377 ng/ml) strains. This extract was subjected to different sepa-

ration techniques to yield four compounds including three isoquinoleine alkaloids and one free sugar. The alkaloids were identified to palmatine (24) (Budavani et al., 1989), jatrorrizine (25) (Budavani et al., 1989) and isocoridinium hydrochloride (26) (Guinaudeau et al., 1983) while the sugar was identified to viboquercitol (27) (Ahmet et al., 1998).



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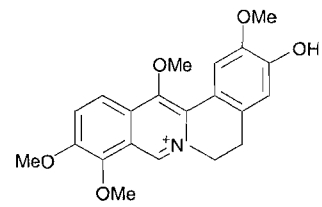

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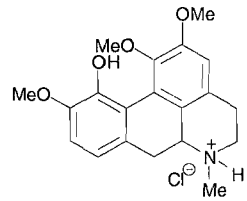

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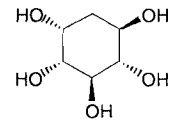

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Table 4. *In vitro* Antimalarial Activity of *Penianthus longifolius* compounds against W-2 and D-6 Clones of *Plasmodium falciparum*

Compounds	*Plasmodium falciparum* strains IC_{50} in ng/ ml	
	Indochina (W-2)	Sierra Leone (D-6)
Palmatine (24)	24.4215	37.3638
Jatrorrizine (25)	67.4211	142.4276
Palmatine (24) + Jatrorrizine (25)	26.6167	37.7054
isocoridinium hydrochloride (26)	>5000	>5000
Viboquercitol (27)	>5000	>5000

From this table 4, it can be denoted that palmatine was highly active follow with jatrorrizine while isocoridinium hydrochloride and viboquercitol were completely inactive.

Conclusion

Several plants are used in traditional medicine for the treatment of malaria. Scientific investigation of these plants is required in view to identify the active chemicals that will lead to development of new drugs. Our own study revealed that investigation with ethnomedicinal approach is more efficient. The three plants that produced the most active extracts conducted to identification of many antiplasmodial compounds, a quassinoid namely ailanthinone been the most active. However, this good activity was discouraged by the high toxicity of the molecule. A study of the structure activity relationship

showed that some chemical modification on the structure could improve the activity and also decrease the toxicity.

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