BIOACTIVE SESQUITERPENES, ALKALOIDS AND FLAVONOIDS FROM THREE ANNONACEOUS SPECIES

T.C. FLEISCHER, Ph.D.
Department of Pharmacognosy, University of Science and Technology, Kumasi.
R. D. WAIGH *PhD*
P. G. WATERMAN *PhD*
Phytochemistry Research Laboratories, Department of Pharmaceutical Sciences, University of Strathclyde, Glasgow G1 1XW, Scotland, UK

ABSTRACT

The stem bark extracts of three species of the Annonaceae family namely Friesodielsia enghiana, Artabotrys stenopetalus and Uvaria mocoli showed toxicity to the brine shrimp Artemia salina. Series of chromatographic fractionations led to the isolation of a wide range of chemically diverse compounds including sesquiterpenes, alkaloids and flavonoids. 25 of these compounds showed various degrees of toxicity to the brine shrimp, over 80% showing $LC_{50} < 100$ µg/ml.

Keywords: *Annonaceae, sesquiterpenes, flavonoids, alkaloids, Artemia salina, cytotoxicity.*

INTRODUCTION

The Annonaceae is one of the largest families of flowering plants occurring in both tropical and subtropical regions of Africa, Asia, Australia and the Americas [1] and has a rich ethnopharmacolgical exposé [2, 3, 4]. It has been shown to be a source of cytotoxic compounds [5], the search of which have led to the isolation of a wide range of bioactive secondary metabolites including flavonoids [6], alkaloids [7], styryl pyrones [8], sterols [9] and acetogenins [10, 11]. We have phytochemically investigated three species namely *Friesodielsia enghiana, Artabotrys stenopetalus* and *Uvaria mocoli*, and in this paper we report on the toxicity of 25 compounds isolated from these species, against the brine shrimp *Artemia salina* A. *Leach*.

PHARMACY

EXPERIMENTAL

Plant material

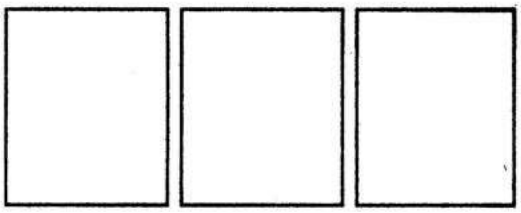
Plant materials tested (stem barks of *Friesodielsia enghiana, Artabotrys stenopetalus* and *Uvaria mocoli*) were collected and identified in Ghana by the Forestry Department, Kumasi, where voucher specimens are kept.

Extraction and isolation

Oven-dried (40°C) powdered stem barks of all the plant species were Soxhlet extracted successively with petrol, $CHCl_3$ and MeOH, except *Uvaria mocoli* which was only extracted initially with EtOAc and later with MeOH. The extracts obtained were evaporated to dryness under reduced pressure at 30°C to give dry extracts. The extracts were initially tested for activity in the brine shrimp and then subjected to a series of different chromatographic techniques including vacuum liquid chromatography, column chromatography and preparative thin layer chromatography to yield the pure compounds [12, 13, 14].

Sample preparation

Test samples were prepared by dissolving them in artificial sea water. Those which were insoluble were dissolved in acetone (2%) or DMSO prior to adding the sea water. For the isoloted compounds, samples were tested at concentrations of 1µg/ml, 10µg/ml, and 100µg/



T.C. Fleischer R.D. Weigh P.G. Waterman

ml; for the crude extracts a 1000μg/ml concentration was included. They were tested in microplates (24 - well plates) in triplicate.

Hatching of the Brine Shrimp

The brine shrimp eggs obtained from Interpet Ltd, Dorking, England, were hatched in a very small tank (the size of a normal soap dish) in artificial sea water prepared from artificial sea salt obtained from Rosewood Pet Products, Shropshire, England. The hatching tank was made up of two compartments. One side which had the eggs was protected from light while the other side was exposed to light to attract the hatched larvae from the dark compartment.

Bioassay

A suspension of the 48 hour old larvae containing 10 - 15 organisms were added to each well containing the test samples, with the aid of a glass pasteur pipette. More artificial sea water was added to make up the volume of the well. The microplates were covered and incubated at the hatching temperature (27 - 29 °C) for 24 hours. The plates were then examined and the motile (living) and the non - motile (dead) larvae were counted with the aid of a hand lens. The LC_{50} values were calculated using GraphPAD Inplot (GraphPAD Software Inc. version 3.14.) The use of potassium dichromate ($K_2Cr_2O_3$) solution with an LC_{50} in the range of 20 to 40 μg/ml [15] as a positive control and a negative control were included. The results were accepted only when the positive control was within the range and the negative control showed mortality of less than 5%, in a concurrent determination [15].

RESULTS AND DISCUSSION

The petrol, $CHCl_3$/EtOAc and MeOH extracts of the stem bark of the three annonaceous species *P. enghiana*, *A. stenopetalus* and *U. mocoli* showed promising activity in the brine shrimp lethality test (LC_{50} < 500 μg/ml) except the MeOH extracts of *A. stenopetalus* and *U. mocoli* (Table 1). Series of chromatographic fractionation of the active extracts resulted in the isolation of a wide range of secondary metabolites which were identified by analysis of their spectral data including UV, IR, 1D- and 2D-NMR, and MS, and for the known compounds by comparison with the literature and in some cases authentic samples [12, 13, 14].

25 chemically diverse secondary metabolites (Table 2) among the isolates were investigated and only the oxoaporphine alkaloid isomoschatoline was not toxic to the brine shrimp (LC_{50} > 1000 μg/ml). Again, apart from the β-sitosterol/stigmasterol mixture and the flavanones 5,7-dimethoxyflavanone and 5,6,7-trimethoxyflavanone, all the compounds tested showed toxicity to the brine shrimp at LC_{50} < 100 μg/ml. The structural activity relationship revealed by the flavanones and oxoaporphines is noteworthy. The results suggested that C-5 hydroxylation of the flavanones is a structural requirement for toxicity in the brine shrimp. This activity appears to be further enhanced by C-2 hydroxylation. Among the unsubstituted ring D oxoaporphine alkaloids, however, C-3 hydroxylation appears to render them non-toxic in the brine shrimp. This may explain why isomoschatoline has not been reported yet as a cytotoxic alkaloid in bioassay - guided fractionation and purification of plants in the Annonaceae and related plant families which produce this group of secondary metabolites. It is significant to note that the β-sitosterol / stigmasterol mixture and bezyl benzoate, the oxoaporphine alkaloids lysicamine and *O*-methylmoschatoline and the sesquiterpene bisabodol have proved cytotoxic to human solid tumour cell lines [8, 10, 16, 17].

The brine shrimp lethality test has been shown to be predictive of cytotxicity [18, 19] and in

REFERENCES

1. Hutchinson J. The Genera of Flowering Plants, Vol. 1, Oxford University Press, London, (1964).
2. Irvine, F. Woody Plants of Ghana, Crown Agents for Overseas Governments and Administration, London, (1961).
3. Burkhill H. M., The useful Plants of West Africa, ed. 2, vol. 1, Royal Botanic Gardens, Kew, (1985).
4. Perry L. M. *Medicinal Plants of East and Southeast Asia: attributed properties and uses*, MIT Press, USA, (1980).
5. Barclay A. S. and Purdue R. E., *Jr. Cancer Treatment Reports* **60**, (8), 1081, (1976).
6. Leboeuf M., Cavé A. Bhaumik P. K., Mukherjee B. and Mukherjee R. *Phytochemistry*, **21**, 2783, (1982).
7. Hufford C. D. and Lasswell W. L., *Jr. J. Org. Chem.*, **41**, 1297, (1976).
8. Harrigan G. G., Bolzani V. DA S., Gunatilaka A. A. L. and Kingston D. G. I. *Phytochemistry*, **36**, 109, (1995).
9. Fang X. P., Anderson J. E., Chang C. J., Fanwick P. E. and McLaughlin J. L. *J. Chem. Soc. Perkin Trans. 1*, 1655, (1990).
10. Jung J. H., Pummangura S., Patarapanich C. and McLaughlin J. L. *Phytochemistry*, **29**, 1667, (1990).
11. Zafra-Polo M. C., Gonzalez M. C., Estornell E., Sahpaz S. and Cortes D. *Phytochemistry*, **42**, 253, (1996).
12. Fleischer T. C., Waigh R. D. and Waterman P. G. *Phytochemistry*, **44**, 315, (1997).
13. Fleischer T. C., Waigh R. D. and Waterman P. G. *J Nat. Prod.*, **60**, 1054, (1997).
14. Fleischer T. C., Waigh R. D. and Waterman P. G. *Phytochemistry*, **47** 1387, (1997).
15. Sam T. W., Toxicity Testing in the Brine Shrimp *Artemia salina*, in Bioactive Natural Products: Detection, Isolation and Structural Elucidation, Colegate S. M. and Molyneux R. J. (Eds), CRC Press, Lonndon, (1993).
16. Borup-Groutchmann I. and Kingston D. G. I. *J Nat. Prod.*, **45**, 102, (1982).
17. Wijeratne E. M. K., Gunatilaka A. A. L., Kingston D. G. I., Haltiwanger R. C. and Eggleston D. S., *Tetrahedron*, **51**, 7877, (1995).
18. Meyer B. N., Ferrigni N. R., Jacobsen L. B., Nichols, D. E. and McLaughlin J. L. *Planta Med* **45**, 31, (1982).
19. Solis N. P., Wright C. W., Anderson M. M., Gupta M. P. and Phillipson J. *Planta Med.* **59**, 250, (1993).

Table 1: Toxicity of crude extracts to *Artemia salina* Leach

Plant species	LC_{50} (μg/ml) (95% Confidence Interval)		
	Petrol Extract	$CHCl_3$/EtOAc Extract	MeOH Extract
Friesodielsia enghiana	12 (7-21)	14 (8-26)	132 (99-199)
Artabotrys stenopetalus	65 (36-110)	93 (69-143)	971 (452-4231)
Uvaria mokoli		123 (9-15)	>1000

Table 2 Toxicity of the isolated compounds to *Artemia salina* Leach

Compound	LC_{50} (μg/ml)	95% C. I. [a]
Sesquiterpenes		
β-bisabodol	12	9 - 26
Gossonorol	25	21 - 28
Pogostol-*O*-methyl ether	52	43 - 65
Artabotrol	92	75 - 121
Oxoaporphine alkaloids		
Lysicamine	28	15 - 67
Isomoschatoline	<1000	
Artabotrine	12	1 - 23
Flavones		
5-Hydroxy-7-methoxy-6-methylflavone	33	28 - 38
5-Hydroxy-7-methoxy-8-methylflavone	42	26 - 67
5-Hydroxy-7-methoxy-6,8-dimethylflavone	30	25 - 37
5-Hydroxy-7-methoxyflavone	19	14 - 21
Flavanones		
5-Hydroxy-7-methoxyflavanone	115	74 - 308
5,7-Dihydroxy-6-methylflavanone	18	13 - 28
5,7-Dimethoxyflavanone[b]	>100	
5,6,7-Trimethoxyflavanone[b]	>100	
2,5-Dihydroxy-7-methoxy-8-methylflavanone[c]	18	9 - 34
2,5-Dihydroxy-7-methoxy-6-methylflavanone[c]	18	9 - 34
2,5-Dihydroxy-7-methoxyflavanone	5	3 - 5
Chalconoids		
2'-Hydroxy-4',6'-dimethoxychalcone	5	0.01 - 9
2'-Hydroxy-4',5',6'-trimethoxychalcone	23	19 - 30
2-Hydroxy-4,5,6-trimethoxydihydrochalcone	51	33 - 76
Others		
Benzoic acid	78	67 - 89
Benzyl benzoate	12	3 - 14
Benzyl 2-hydroxybenzoate	23	21 - 25
β-Sitosterol/stigmasterol [c]	101	82 - 134

[a] C. I. = Confidence Interval
[b] Not toxic at 100μg/ml
[c] Tested as a mixture