A PRELIMINARY STUDY OF CHEMISTRY AND TOXICOLOGY OF A NATURAL ANTIPROTOZOAL AGENT

A thesis submitted for the degree of
Master of Rural Science of the University of New England

by

RUSMANA WIJAYA SETIA NINGRAT

DEPARTMENT OF BIOCHEMISTRY, MICROBIOLOGY AND NUTRITION
THE UNIVERSITY OF NEW ENGLAND ARMIDALE NSW 2351
AUSTRALIA

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PREFACE

The studies presented in this thesis were completed by the author while a post graduate student in the Department of Biochemistry, Microbiology and Nutrition, the Faculty of Science, the University of New England, Armidale, N.S.W., Australia. Assistance given by other persons is indicated in the text or in the list of acknowledgements. All references cited are included in a bibliography. The work is otherwise original.

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I certify that the substances of this thesis has not already been submitted for any degree and is not being currently submitted for any other degree.

I certify that any help received in preparing this thesis, and all sources used, have been acknowledged in this thesis.

March, 1994 *Rusmana Wijaya Setia Ningrat*

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SUMMARY

A survey of some tree forage supplements with potential to manipulate rumen protozoa found that *Enterolobium cyclocarpum* Griseb. showed antiprotozoal activity (Leng *et al.*, 1992). However, there is no further information available on the active agent(s) present in this plant.

Therefore, a preliminary study on the chemistry and toxicology of the active agent present in the extract of *Enterolobium* leaf was carried out. Working on the hypothesis that the active agent was a saponin, the active agent was isolated by maceration of the water-soluble compounds from the dried leaves, followed by partitioning between water and n-butanol layer with the butanol layer containing the active fraction. Further fractionation of the leaf extracts was guided by two bioassays, haemolytic assay and antiprotozoal assay.

A haemolytic assay using sheep erythrocytes has been developed to test the presence of saponins. The purified fractions showed strong haemolytic activity, although comparative studies with white saponin and Alkanate 3SL3 (sodium lauryl diethoxysulphate) indicated that haemolytic activity may not reflect a parallel ability to lyse rumen protozoa. White saponin, the most potent agent observed to lyse red blood cells, has been found to have no activity on protozoa, whereas Alkanate 3SL3, the second in these comparative studies, has been found to effectively eliminate protozoa from the rumen of lambs (Burggraaf, 1980). Therefore, the antiprotozoal assay using rumen protozoa was developed The antiprotozoal assays were conducted under two different conditions, aerobic and anaerobic systems.

Recent *in vitro* results suggest that those purified fractions exhibiting a pronounced ability to immobilise rumen protozoa tend to produce high lytic activity with these cells. Study on the possible synergistic interaction effects among components in the leaf extract indicated that activity was not affected after fractionation. It appears that the results are consistent within experiments but not between experiments and this may be due to the number of protozoa fluctuating widely between samples from the donor animal.

Gas-Liquid Chromatography analysis of trimethylsilyl derivatives of monosaccharides liberated by acid hydrolysis of leaf components was employed to help elucidate the structure of the active agents. The proposed structures of the active agents were reported. Since the nature of the aglycone was not studied in this project, it was assumed that the analysed fractions are composed of saponins containing the single aglycone machaerinic acid lactone, as identified in *Enterolobium contortisiliquum* (Delgado *et al.*, 1984). It appears that the structure of the active agent is unlikely to be substituted with more than one sugar. Glucose seems predominant followed by rhamnose, arabinose and xylose, while galactose is not present in all fractions.

In summary, the purified fractions obtained after extensive reverse-phase HPLC were, in general, still unresolved mixtures of saponins.

ABBREVIATIONS

Ara	Arabinose
ATP	Adenosine triphosphate
DHPS	Dihydropteroate synthetase
Gal	Galactose
GLC	Gas Liquid Chromatography
Glc	Glucose
HPLC	High Performance Liquid Chromatography
MAL	Machaerinic acid lactone
NMR	Nuclear Magnetic Resonance
PABA	Para amino benzoic acid
PExt	Partitioned extract (crude butanol extract)
Rha	Rhamnose
TMS	Trimethylsilylation
tRNA	transport Ribonucleic Acid
VFA	Volatile Fatty Acids
Xyl	Xylose

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