

## Constituents of some Asian medicinal plants

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**Abstract:** The results of some collaborative projects with Southeast Asian chemists are reported. The Thai medicinal plant *Schefflera lucantha* used against asthma attack has yielded three saponins with bronchodilatory effect. Their structures have been shown to **1**, **2** and **3**. Cyclopeptide alkaloids have been isolated from two Indonesian *Antidesma* species and their structures determined as **5**, **6**, **7** and **8**.

In the countries of Southeast Asia research on bioactive natural products is encouraged and supported by the UNESCO Regional Network for the Chemistry of Natural Products in S.E. Asia. Further support has come from the Australian-funded Network for the Chemistry of Biologically Important Natural Products through the facilitation of collaboration between Australian chemists and chemists in the Region. The emphasis has been on helping chemists in developing countries to work on their own biological material, at their own pace. Two such collaborative projects involving the University of Sydney have been with Silapakorn and Chiang Mai Universities, Thailand, on the saponins of *Schefflera lucantha* and with Andalas University, Padang, Sumatra, Indonesia, on the alkaloids of *Antidesma* species,

*Schefflera lucantha* Viguier (local name: hanumarn prasarnguy) is an ornamental plant which grows to about 2-3 m in height. Its leaves are used in Thai traditional medicine for relieving asthmatic attack, by making an extract with hot water. Pharmacological studies indicated that the aqueous extract relaxed smooth muscle of the isolated guinea-pig tracheal chain. Partitioning of a total concentrate of the ethanol extract of leaves between ethyl acetate and water gave an aqueous fraction possessing the relevant activity. This activity was transferred to a *n*-butanol extract of the aqueous fraction. Column chromatography of the *n*-butanol extract on Amberlite XAD-2 resin with water and then water/methanol mixtures gave a purified active fraction. This fraction was further purified by chromatography on XAD-7 to give a single spot (t.l.c.) saponin fraction which showed strong biological activity. This was a mixture of glycosides; hydrolysis gave oleanolic and betulinic acids, and a mixture of D-glucuronic acid, D-glucose, D-xylose and L-rhamnose.

The saponin mixture was separated by preparative h.p.l.c. to give glycoside A, B and C. The structure of each glycoside was determined by spectroscopic evidence and hydrolysis to an aglycone and sugars.

Glycoside A, C<sub>47</sub>H<sub>76</sub>O<sub>18</sub>, showed a protonated molecular ion at 941 in the CI (FD) mass spectrum. Prominent fragment ions at *m/z* 794, 632 and 456 corresponded to the sequential loss of a deoxyhexose, a hexose and a hexuronic acid, suggesting that the sugar residue was a linear chain trisaccharide.

The  $^{13}\text{C}$  n.m.r. spectrum showed signals for three anomeric carbons ( $\delta$  102.2, 102.2, and 105.5) and for two carboxyl groups ( $\delta$  173.3 and 179.0). A peak at  $\delta$  90.1 showing significant glycosidation shift was indicative of the sugar moiety being attached to the aglycone at C3. Methanolysis of glycoside A gave betulinic acid and D-glucuronic acid D-glucose and L-rhamnose (as methyl glycosides).

The connectivity of the sugar sequence was confirmed by the  $^1\text{H}$  n.m.r. spectrum of the octaacetate dimethyl ester derivative **4**. The resonances of the anomeric protons H1', H1'' and H1''' were clearly seen in the 600 MHz spectrum. A DQF COSY spectrum (400 MHz) showed that in the case of H1' and H1'', the vicinally coupled H2' and H2'' respectively resonated at  $\delta$  4.01 and 3.62, and had not suffered acetylation deshielding. This indicated that glycoside A was 3-O-[ $\alpha$ -L-rhamnopyranosyl(1 $\rightarrow$ 2)- $\beta$ -D-glucopyranosyl(1 $\rightarrow$ 2)- $\beta$ -D-glucuronopyranosyl]betulinic acid (**1**).

In a similar fashion glycoside B was shown to be 3-O-[ $\alpha$ -L-rhamnopyranosyl(1 $\rightarrow$ 2)- $\beta$ -D-xylopyranosyl(1 $\rightarrow$ 2)- $\beta$ -D-glucuronopyranosyl]betulinic acid **2** and glycoside C as 3-O-[ $\alpha$ -L-rhamnopyranosyl(1 $\rightarrow$ 2)- $\beta$ -D-glucopyranosyl(1 $\rightarrow$ 2)- $\beta$ -D-glucuronopyranosyl]oleanolic acid **3** (**1**).

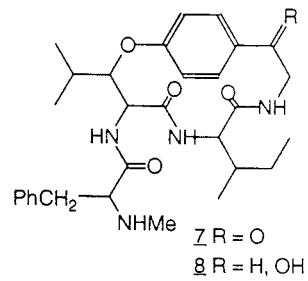
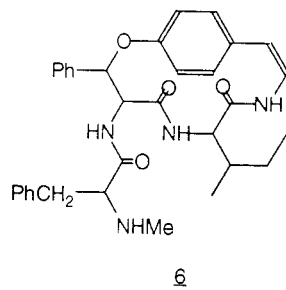
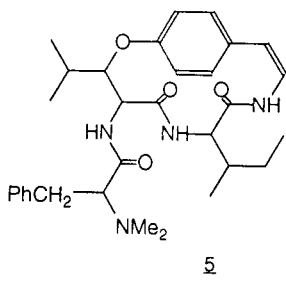
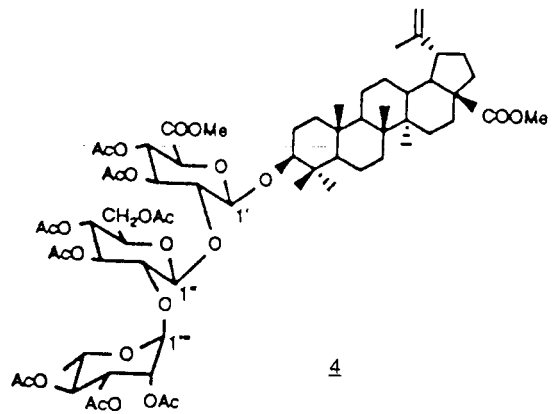
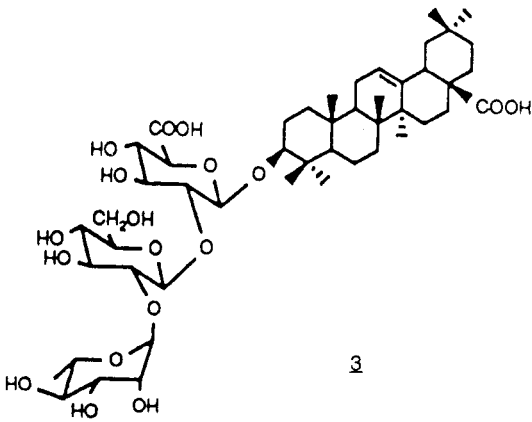
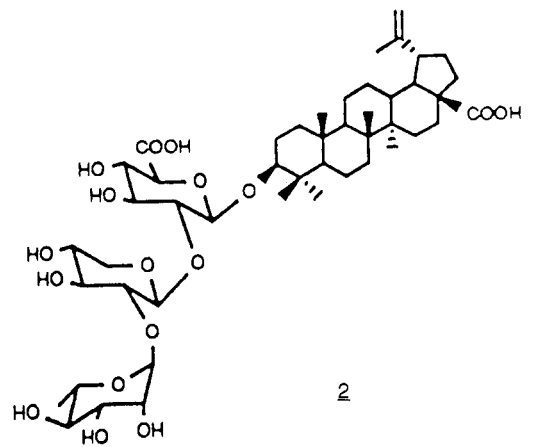
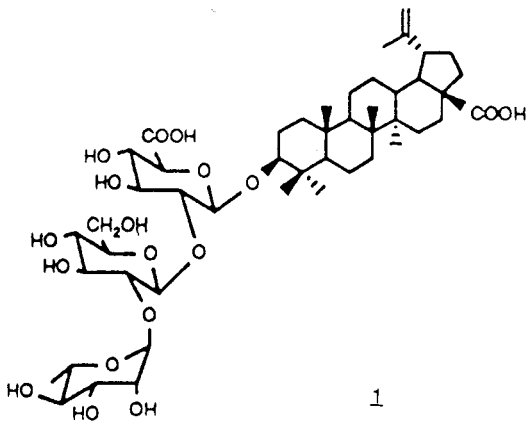
Saponins derived from oleanolic acid are quite widely spread, but saponins based on betulinic acid are rather rare, and have been reported mainly from other species of *Schefflera*. The biological properties of the saponins are being examined further.

*Antidesma montanum* (family, Euphorbiaceae) is a small tree found in southern China, the Malaysian Peninsula, and Indonesia. It is reported to have medicinal value in Malaysia. In West Sumatra it is used by some traditional healers and the tree is well-known to children because the fruit of the wild plant is edible. Although there is no record of the isolation of alkaloids from *Antidesma* species, *A. montanum* was found to give a positive Mayer's test during a phytochemical survey in West Sumatra and this prompted the present work.

Conventional extraction of the leaves and terminal branches yielded a small quantity of crude base which contained two major components (by t.l.c.). Chromatographic separation proved difficult, but a procedure relying on basicity differences proved satisfactory: Partitioning of the base fraction between ethyl acetate and aqueous tartaric acid gave a good separation of the two major components, with one major alkaloid in each phase, and further chromatography on silica gel then yielded two pure alkaloids - AM-1 and AM-2.

The two alkaloids were shown by analysis of the  $^1\text{H}$  and  $^{13}\text{C}$  n.m.r. spectra and the mass spectra to have the cyclopeptide structures **5** and **6** respectively (**2**). The relative structure **5** has already been assigned to myrianthine B, isolated from *Myrianthus arboreus* P. Beav. (Urticaceae) and the structure **6** to aralionine B. However there was no satisfactory agreement between the physical constants of AM-1 and AM-2 and those reported for myrianthine B and aralionine B respectively. Differences in absolute configurations of constituent amino acids giving rise to diastereoisomerism could underlie the discrepancies in physical properties.

Because of these problems the chiralities of the intact amino acid residues were determined. To do this we have utilized a g.l.c. method involving analysis of derivatized amino acids produced by acid hydrolysis, on a chiral capillary column (Chirasil-Val) (**3**). Derivatization was in the form of the *N*-pentafluoropropionyl, isopropyl ester. In this way it was a simple matter to identify the isoleucine as being in the *L*- absolute stereochemical form, as was also the *N*-methylphenylalanine produced from AM-2. Hydrolysis of AM-1 produces *N,N*-dimethylphenylalanine, which cannot be analysed directly for the simple reason that it cannot be *N*-perfluoroacylated. Methods of *N*-demethylation were therefore examined. The von Braun, cyanogen bromide, method was unsatisfactory, but the method utilizing



ethyl chloroformate gave good results. The complete sequence of reactions of hydrolysis, demethylation and derivatisation can be performed in the one pot, on a sub-milligram scale. In this way the configuration of the *N,N*-dimethyl-phenylalanine unit in AM-1 was determined also to be L.

Examination of the leaves of the related species, *A. tetrandrum*, yielded an alkaloid fraction which could not be separated by normal chromatographic methods. By using h.p.l.c. two bases were obtained. The  $^1\text{H}$  n.m.r. spectra of the new alkaloids **7** and **8** had similar features to those of **5** and **6** except that the two proton pattern of the amino styrene system was absent. In its place, in the spectrum of **7**, was an AB pattern with a large (17 Hz) geminal coupling (C=O effect), with vicinal coupling also with the adjacent amide NH. In the spectrum of **8** an ABX pattern was present from the alcohol methine and the adjoining methylene. It is not clear whether these compounds, which are generally not common, can be considered as precursors of the styryl pattern, or in fact arise subsequently. Further chemical characterization is in progress.

#### Collaborators:

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##### 2. *Antidesma* spp.

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