Bioactive Principles from Folkloric Anti-Neoplastic Plants and from BioproSpecting the Malaysian Forest

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Abstract: In contrast to the rapid economic development in S.E. Asia, scientific studies on the region's flora have largely been neglected in relative terms. Plants especially those of use in folklore medicine, need to be continually studied for proof of their efficacy or otherwise. Likewise the tropical forest in this region remains a genetic treasure-house for bioprospecting for natural products with bioactive activity. Even though there are international agreements and protocols, biodiversity has in recent times been the object of exploitation or be decimated by natural disasters, conservation efforts have not received the adequate international support or funding and it is increasingly evident that nations have to be self reliant in their efforts for the conservation and the development or promotion of beneficial uses of their bioresources. Although detail phytochemical and pharmacological studies have remained painfully slow, better instrumentation has allowed for a rich harvest of new natural products and bioactive substances. Novel natural products and bioactive compounds especially with cytotoxic and insecticidal activities, e.g. new prenylated xanthones, biflavonoids and coumarin derivatives, have been characterized from plants of the family Guttiferae. Biogenetically interesting compounds, e.g. a novel seco-trisnor-oleanane and seco-benzyltetrahydroisoquinoline, were isolated from from Calophyllum gracilipes and Polyalthia insignis, respectively. Many substituted coumarins of potential against HIV protease activity were isolated Calophyllum plants including Calophyllum teysmannii. Plants from the family Annonaceae provide many cytotoxic and insecticidal compounds such as styrylpyrone derivatives, acetogenins and aporphine derivatives. From among the folklore plants, vaguely referred to as for treatment of neoplastic conditions, the strongly cytotoxic 5,6-dihydroxyindole from Rhaphidophora korthalsii was isolated by bioassay-guided fractionation.
INTRODUCTION

The quest for bioactive natural products from the S.E. Asian region has been going on for decades and a wide variety of bioactive compounds with potential has been discovered. However the number of new drugs developed, e.g. for diseases such as cancer and AIDS or for combating tropical diseases, has in general been quite disappointing. The battle against cancer has been painfully slow, e.g. with the exception of some useful natural products for use in chemotherapy such as vincristine, vinblastine, taxol, camptothecine derivatives, and some anticancer antibiotics, the development of new effective drugs has not been very encouraging. Much of this pessimism has been rooted in our failure to understand the nature and mechanism of cancer development. Previous efforts have been focussed essentially on spindle poisons, i.e. on the disruption of microtubule assembly or disassembly, which of course is important in disrupting rapid tumour growth. Even the recent successful use of taxol and its derivatives has depended on this mechanism albeit being slightly different in promoting polymerisation of microtubules, thereby disrupting cell proliferation. Recent discoveries have provided more hope as more mechanisms of action against cancer cells become known. For example, natural products can be used as angiogenesis inhibitors, apoptosis inducers, cell-cycle regulators (cyclins) and telomerase inhibitors.

Our foray into the area of bioactive natural products has been incidental rather than intensive because of limited resources.1-3 Some of our earlier compounds of interest have been the dimeric indole alkaloids4,5 from Uncaria callophylla. Callophylline (1) from this plant was notably cytotoxic but, although like vincristine it is a dimeric indole alkaloid, structurally it was different. Conophyllidine (2)5, another dimeric indole from the common tropical plant Tabernaemontanae divaricata, is also a promising alkaloid for development. Simpler alkaloids derived from indoles such as rhazinilam (3)6 isolated from Leuconotis griffithii and some Malaysian Kopsia species are also spindle poisons. A number of such "ring-opened" indoles (e.g. 4 and 5) are also provided by two Leuconotis species6 but leuconolam (5) was however inactive.
The search for bioactive principles is part of our continuing interest in finding useful natural products from the flora of the S.E. Asian region.\textsuperscript{1-3} This requires that we examine as many of the forest plants as possible including plants used in traditional medicines for their chemistry as well as their bioactivity. Phytochemical screening combined with bioassays of the plants are the first steps towards the discovery of useful natural products for drug development and other applications.

**RESULTS AND DISCUSSION**

Previous reports\textsuperscript{1-3} have described many phytochemical studies and more than 1,600 Malaysian plants have been recorded. These efforts have covered only a small part of the available plant biodiversity in the S.E. Asian region, considering that Malaysia alone is home to at least 15,000 species of flowering plants. Our plant collection effort has been led by our botany collaborators and species collections somewhat reflect their bias. Nevertheless a wide selection of plants has been collected for phytochemical and selected biological assays.

**Bioassays**

Among the large number of plants, many species of the Annonaceae family have provided natural products which have cytotoxic activity.\textsuperscript{7,8} Several species of the *Goniothalamus* are outstanding in their phytochemistry and biological activity from the variety of specimens studied.\textsuperscript{1,2}
Cytotoxicity assays were on a few cell lines as well as on the larvae of *Aedes aegypti*. Crude and fractionated extracts with positive assays at ED$_{50} < 25$ mg/mL or LC$_{50} < 300$ mg/mL were examined further. Typical results$^9,10$ are provided by the group of compounds which are derivatives of styrlypyrones and these are given in the Table 1.

The group of compounds comprising goniothalamin (6a) and related compounds (6 - 9) have been isolated previously or prepared semisynthetically.$^7$-$^{14}$ From the limited number of styrlypyrones and related derivatives included in the Table, it appears that the structure and activity relationships are not particularly pronounced for this series; in general this class of compounds were mostly moderately cytotoxic. Aristolactam (10), also isolated from plants of the Annonaceae family, also showed promising activity. Other classes of cytotoxic compounds came from coumarin derivatives$^{12}$-$^{14}$ (e.g. calanone 13a) and a xanthone derivative (e.g. pyranoamentoflavone 14a).$^{11}$

Table 1. Some Bioactive Compounds$^9,10$

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cell Lines (Cytotoxicity)$^a$</th>
<th>Larvicidal Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P388</td>
<td>B16/F1</td>
</tr>
<tr>
<td>Goniothalamin (6a)</td>
<td>0.75</td>
<td>1.1</td>
</tr>
<tr>
<td>Goniothalamin epoxide (6b)</td>
<td>&gt;4</td>
<td>3.1</td>
</tr>
<tr>
<td>Isogoniothalamin epoxide (6c)</td>
<td>0.8</td>
<td>&gt;4</td>
</tr>
<tr>
<td>5α-Hydroxygoniothalamin (6d)</td>
<td>&gt;4</td>
<td>4.0</td>
</tr>
<tr>
<td>5β-Hydroxygoniothalamin (6e)</td>
<td>1.3</td>
<td>3.5</td>
</tr>
<tr>
<td>5α-Acetoxygoniothalamin (6f)</td>
<td>2.0</td>
<td>0.9</td>
</tr>
<tr>
<td>5β-Acetoxygoniothalamin (6g)</td>
<td>0.7</td>
<td>4.0</td>
</tr>
<tr>
<td>(-)-Iso-5-deoxygoniopyrrole (7)</td>
<td>&gt;4</td>
<td>0.9</td>
</tr>
<tr>
<td>(+)-Goniothalenol (8)</td>
<td>&gt;4</td>
<td>4.0</td>
</tr>
<tr>
<td>Goniobutenolides E&amp;Z (9a)</td>
<td>6</td>
<td>22</td>
</tr>
<tr>
<td>Aristolactam AIII (10)</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>Aristolactam BII (11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ouregidione (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calanone (13a)</td>
<td>26</td>
<td>40</td>
</tr>
<tr>
<td>Pyranoamentoflavone (14a)</td>
<td>5</td>
<td>16</td>
</tr>
</tbody>
</table>

$^a$ED$_{50}$ values; compounds are cytotoxic for values < 40 mg/mL.
Structural Elucidation

Structure elucidation for pure isolated compounds is less of a problem in recent times if NMR and mass spectral instruments are readily available. Previously, e.g. with compounds (1,3-5), only one-dimensional (proton and carbon, and DEPT) NMR spectra was available for structure determination. With the advent of two-dimensional NMR techniques structure elucidation is normally a routine exercise if NMR time is available. Once the molecular weight can be determined by high resolution mass spectroscopy, a 5 mg-sample is usually sufficient to carry out NMR experiments using a 500 MHz NMR instrument. When required 2D-HMQC, COSY, NOESY, HMBC and NOE-difference spectra were routinely employed for complete structure determination. Long range H-C correlations were provided by HMBC experiments while through space proximal groups were from NOESY and NOE-difference experiments. When there was still insufficient NMR data to confirm the structure or when absolute and relative configurations were required, single X-ray diffraction was carried out. Typically, for structure determination the example of the novel seco-tris-nor-triterpene (17) may be illustrated as shown below. NMR spectra, NOE effects and HMBC being particularly useful, provided connectivity patterns of the carbons and hydrogens as shown. The stereochemistry of the D/E junction was however not evident from the NMR spectra and an X-ray structure determination had to be carried out. The structure as shown shows a novel ring-A degraded oleanane skeleton (17). Novel seco-isoquinoline alkaloids (18) from Polyalthia insignis were also determined from their NMR spectra; they are likely derived from isoquinoline alkaloid precursors.15

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Among the plants from Annonaceae, *G. borneensis* was noteworthy in providing a large number of bioactive compounds, which were notably styrylpyrone derivatives. The variety of compounds can rival those reported from another plant of the same genus from Thailand, i.e. *G. giganteus*. The biogenesis of styrylpyrone derivatives may be illustrated in Fig. 1. Similar compounds were...
reported from *G. giganteous* which also provided additional compounds such as gonioheptolides which are related eight-ring lactones.\(^{16}\)

Also produced from the Annonaceous plants are acetogenins\(^8\)-\(^{15}\) which are more potent cytotoxic compounds. Some typical examples were annonacin (15) (from *G. velutinus*, *G. malayanus* and *G. dolichocarpus*) and disepalin (16) from *Disepalum anomalum*.\(^7\),\(^8\) Aporphinoid and related alkaloids were also produced from some *Goniothalamus* species and some novel ones were aristolactam B(III) (11) and ouregidione (12).\(^10\) Aristolactams (10, 11 and others) from *G. borneensis* have also been found to be a useful group of cytotoxic compounds.\(^10\) *G. borneensis* provided several bioactive compounds including goniothalenol (8), hydroxylated goniothalamins (9c - 9e), pinocembrin and several other compounds related to styrylpyrones as illustrated in Fig. 1.

Several coumarin derivatives including calanone (13a) from *C. teysmannii* have been tested also to provide minor bioactivity against cancer cell lines. In fact this plant has three varieties and provided a rich harvest of coumarin-type natural products (13b - 13m) as shown. Among several forest plants collected for preliminary screening, betulinic acid (19) has appeared to be a potent but minor component in *Calophyllum gracipes*, *C. blancoi* and *C. teysmannii*. 19 is a strongly cytotoxic triterpene,\(^17\) reportedly useful for the treatment of melanoma.
Novel biflavonoids have also been isolated from *Calophyllum* species, among which 20 and 21 are novel. Apart from forest plants, common folkloric plants have also come into use in this region. Several compounds have been found by several interested groups in *Typhonium divaricata* which has been among the well utilized medicinal plants for cancer treatment. In Singapore there had also been widespread use of another plant locally referred as "Dragon tail" or *Rhaphidophora korthalsii* (revised to *Epipremnum pinnatum*). It is of interest that the active constituent is a small but unstable molecule and has been characterised by us as 5,6-dihydroxyindole (22a). Its isolation presented special problems because of its rapid oxidation to black polymeric materials. However the use rapid vacuum chromatography techniques has allowed the compound to be isolated and to be further stabilized as the diacetate (22b). The natural and synthetic 5,6-dihydroxyindole are identical and undergoes oxidation under physiological conditions. Several derivatives have been made and those with better stability and activity are presently undergoing tests.

Studies of plants of the S.E. Asian region, especially those of use in folklore medicine, will remain to be fruitful; the tropical rainforest is a genetic treasure-house for bioprospecting for natural products with bioactive principles. However, with each passing year, we find that the tropical forest biodiversity is continually being threatened by rapid economic development as well as by natural disasters. Even though there are international agreements and protocols, biodiversity has in recent times been the object of exploitation or has been threatened to be decimated by fires and other natural disasters. The call for conservation efforts need to be renewed as adequate international support or funding has not been forthcoming. It is increasingly evident that nations have to be self reliant in their efforts for the conservation and the development/promotion of beneficial uses of their bioresources.

REFERENCES