Antimitotic and Cytotoxic Activities of Guattegaumerine, a Bisbenzylisoquinoline Alkaloid

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Guattegaumerine (**1**) was isolated in our laboratory from the bark of *Guatteria gaumeri* Greenman (1), a medicinal plant, called "E(k)lemuy" or "Yumel" in Yucatán (South-East Mexico) (2). Guattegaumerine is a partly methylated bisbenzylisoquinoline alkaloid and is closely related to thalicarpine (**2**) which has antitumoral activities, principally against the Walker intramuscular carcinosarcoma 256 in the rat (3, 4). We thought it would be interesting to see whether guattegaumerine has cytotoxic activities. Thus, we performed some preliminary microtests and the results were confirmed by more precise experiments, performed on some types of cells.

Guattegaumerine was obtained as described earlier (1). The preliminary microtests were carried out as mentioned previously (5) on:

- cultured B16 melanoma cells, derived from C57 BL mouse melanoma;
- cultured Flow 2002 cells, line derived from normal embryonic human lungs;
- cultured HeLa cells, line derived from a human carcinoma;
- cultured L 1210 cells, line derived from DB A/2 mouse ascites tumor.

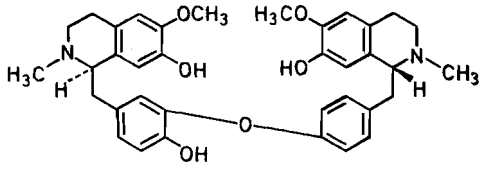
The alkaloid was added to the culture medium at various concentrations 24 hours after starting cultivation.

More precise antimitotic activity tests were performed on slides, as described previously (6) using B16 melanoma and Flow 2002 cells. After 72 hours of treatment, the cells were fixed and stained by a Feulgen reaction for cytological analysis (light microscopy). Mitotic activity or degree of cell death was expressed as mitotic or pycnotic indexes (number of cells in mitosis or pycnosis for 1,000 cells). The percentages of the mitotic phases were calculated for detecting eventual mitotic disturbances. Mean indexes were compared with Student's t-test.

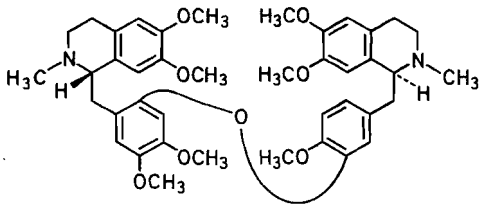
The results are shown in Tables I and II. Preliminary microtests show that guattegaumerine exerts a strong activity at 10 µg/ml not only on L 1210, a quite sensitive tumor, but also on B16 melanoma, which is a relatively resistant tumor (Table I). The activity on HeLa and 2002 cells is lower and this fact can be of interest because 2002 cells are in fact normal human cells.

More precise tests were performed in order to confirm these results (Table II). They showed that guattegaumerine exerts some activity even at concentrations below 5 µg/ml on B16 melanoma but it is more than two times less toxic on normal human cells.

The comparison of the activity of guattegaumerine with thalicarpine is difficult because we did not have the opportunity



1 Guattegaumerine



2 Thalicarpine

Table I. Preliminary microtests

Dose	B16	L 1210	2002	HeLa
1 µg/ml	0	0	0	0
10 µg/ml	+++	+++	+	+

0 = no effect;+ = visible effect after 72 h treatment; +++ nearly all the cells are dead after 24 h treatment.

Table II. Tests on slides

Treatment (µg/ml)	Mitotic index (‰)	P	Pycnotic index (‰)	P
Melanoma B16				
control	15.7	–	32	–
1	15.7	10 (n.s.)*	50.5	95
5	0	–	852.5	> 99.9
10	0	–	884	> 99.9
Flow 2002				
control	10.8	–	2.5	–
1	12.9	60 (n.s.)*	2.3	10 (n.s.)*
5	7.6	85	4.3	80
10	1.3	99	33.5	> 99.9

* n.s. = not significant

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to test them in the same experiments; however, the *in vitro* activity of thalicarpine on KB cells is in the same dose range (4).

We now plan to perform further experiments with guattegaumerine by inoculation into animals bearing an experimental tumor.

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Marine Natural Products; II. Chemical Constituents of Red Alga *Botryocladia leptopoda*

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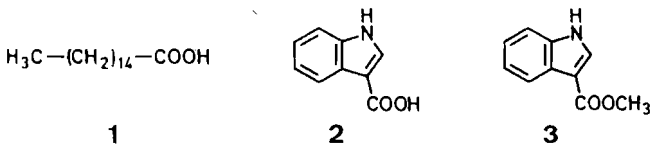
Phytoplankton organisms and higher algae are regarded as the primary producers in the oceans and have led to a wide range of bioactive substances (1). The crude methanolic extract of *Botryocladia leptopoda* showed strong antibacterial activity against *Bacillus subtillis* and *Bacillus megaterium* (2). We have previously reported 3-formylindole from the red alga *Botryocladia leptopoda* (3). Our continued studies have now resulted in the isolation of some known organic compounds from the same source, which have not been reported so far from this alga.

Results and Discussion

The mass spectrum of compound **1** (m.p. 58–60° C) showed a molecular ion peak at *m/z* = 256 (42 %) corresponding to the molecular formula $C_{16}H_{32}O_2$. Other important peaks were present at *m/z* (rel. intens.) = 213 (30), 185 (28), 171 (28), 157 (57), 129 (48), 97 (52), 73 (98), 59 (100). The ^{1}H-NMR in $CDCl_3$ exhibited the peaks at δ = 0.88 (t, CH_3), 1.26 (s, CH_2), 2.34 ppm (t, CH_2-COO-). From the mass and NMR, the structure of compound **1** was assigned as hexadecanoic acid. The terminal COOH group was shown by the formation of methyl ester on treatment with diazomethane. The further confirmation of the structure was carried out through ^{13}C-NMR spectrum which shows the signals: 14.1 (C-1), 22.71 (C-2), 24.7 (C-3), 24.7 (C-4), 27.26 (C-5), 29.12 (C-6), 29.12 (C-7), 29.28 (C-9), 29.38 (C-10), 29.47 (C-11), 29.70 (C-12), 29.70 (C-13), 31.96 (C-14), 34.10 (C-15), 180.05 (C-16) ppm.

Compound **2** (m.p. 198–200° C) showed a molecular ion peak at *m/z* = 161 (82 %) corresponding to the molecular formula $C_9H_7NO_2$. Other peaks appeared at *m/z* (rel. intens.) = 144 (100), 116 (35), 83 (52). The UV spectrum showed the characteristic indole skeleton with absorptions at λ_{max} = 222, 240 (sh), 280, 286 nm. The ^{1}H-NMR spectrum exhibited a multiplet in the region between δ = 12–7.20 assigned to H-5 and H-6, two double doublet at δ = 7.41 (*J* = 7.5 Hz, 3 Hz) and 8.07 ppm (*J* = 7.5 Hz, 3 Hz), were assigned to H-4 and H-7, respectively. A sharp singlet at δ = 7.90 ppm was due to H-2. From these spectroscopic studies the structure of compound **2** was assigned indole-3-carboxylic acid. The structure was further confirmed by ^{13}C-NMR data, which showed the following signals 138.2 (C-2), 110.0 (C-3), 123.43 (C-4), 122.17 (C-5), 122.17 (C-6), 112.78 (C-7), 133.0 (C-8), 128.56 (C-9), 171.0 (C-10) ppm.

The mass spectrum of compound **3** (m.p. 140° C) showed the molecular ion peak at *m/z* = 175 (35 %), corresponding to the molecular formula $C_{10}H_9NO_2$. The ^{1}H-NMR spectrum showed a singlet



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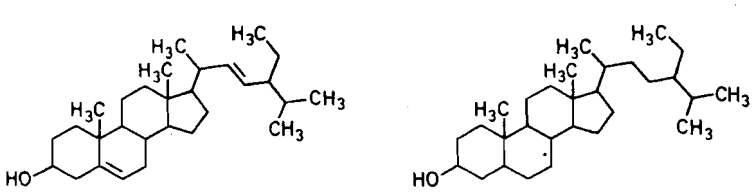

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