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CYTOTOXIC AND ANTITUMOR ACTIVITY OF VISMIONES ISOLATED FROM VISMIEAE

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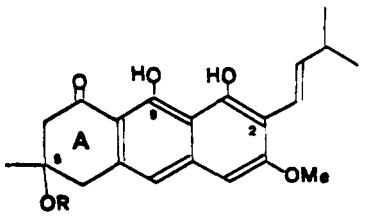
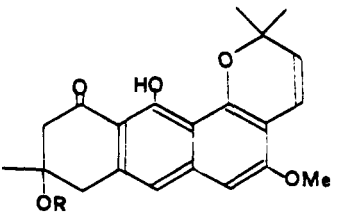
Recently many South American *Vismia* species and African *Psorospermum* species belonging to the same tribe (Vismieae) of Hypericoideae have been examined resulting in the isolation, among other things, of 12 vismiones, whose occurrence is limited to this tribe (1,2).

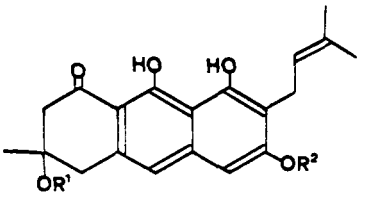
The isolated vismiones (**1-12**, Figure 1) are characterized by a tetrahydroanthracene nucleus with a non-aromatic A ring and differ in the C-6 substituent (OH or OAc), in the C-3 substituent (OH, OMe, OPr, or O-geranyl), and in the C-2 side chain, which may be constituted by mono- or di-prenyl (only in visione G, **12**, the side chain is at C-10) (1). From the chemotaxonomic point of



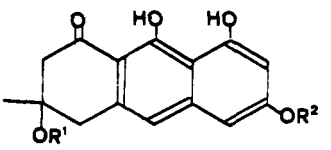
		R
1	vismione A	Ac
2	deacetylvismione A	H



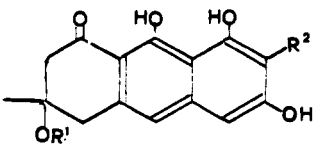
		R
3	acetylvismione B	Ac
4	vismione B	H



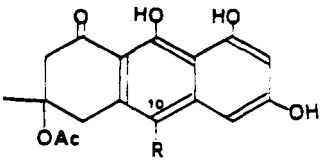
		R^1	R^2
5	vismione C	Ac	H
6	vismione E	H	Me



		R^1	R^2
7	acetylvismione D	Ac	geranyl
8	vismione D	H	geranyl
9	vismione H	Ac	



		R^1	R^2
10	acetylvismione F	Ac	geranyl
11	vismione F	H	geranyl



		R
12	vismione G	geranyl

FIGURE 1. Structure of vismiones.

view, it should be noted that vismiones with a *five-carbon-atom* side chain at C-2 have been mainly found in *Vismia* species, while those with a geranyl chain have been isolated only from *Psorospermum* species.

Vismiones and other secondary metabolites from Vismieae exhibited a good antifeedant activity against Lepidoptera larvae and *Locusta migratoria* (3) but no antibacterial or antifungal activity.[1] This paper will deal with cytotoxicity of vismiones and preliminary data on the in vivo antitumor activity of vismione A (**1**, NSC 339659).

Among the prenylated anthranoids (such as ferruginins, prenylated anthrones or anthraquinones) which we have isolated from Visimieae and tested in vitro, only the vismiones display a significant cytotoxicity against different tumor cell lines (4,5).

The National Cancer Institute (NCI), USA, has used 9KB (Eagle) cell cultures (6) as an antitumor assay for screening plant extracts since 1969 (7) and has played an important role in the discovery of antitumor agents from higher plants (8). However, activity against KB cells does not always correlate with an in vivo activity against experimental tumors, such as ascitic P-388 murine leukemia (9).

In our laboratories we have also adopted two different murine P-388 leukemia cell lines, one sensitive (P-388/S) and one resistant (P-388/R) to doxorubicin, for the primary screening of natural products from fermentation broths or plant extracts. The efficacy of vismiones in inhibiting the growth of 9 KB, P-388/S, and P-388/R cell lines is reported in Table 1. The data show that only in vismione D and F can the presence of a C-6 acetoxy group be correlated with an increased efficacy when compared with the corresponding C-6 hydroxylated compounds. Another interesting finding is that compounds **3** and **4** appear to be slightly more effective against P-388 cells resistant to doxorubicin (Adriamycin®, one of the most active and clinically used antitumor agents) (10) than against doxorubicin sensitive P-388 cells.

TABLE 1. Effect of Vismiones and Doxorubicin on the Growth of 9 KB, P-388/S, and P-388/R Cell Cultures[a]

Vismione	ID_{50} (μg/ml)		
	KB	P-388/S	P-388/R
A (**1**)	0.25	0.45	1.1
Deacetyl A (**2**)	0.7	0.4	0.3
Acetyl B (**3**)	42.0	5.0	2.0
B (**4**)	10.0	10.0	3.0
C (**5**)	2.1	n.d.[b]	n.d.
Acetyl D (**7**)	5.0	1.8	n.d.
D (**8**)	14.0	14.0	18.0
Acetyl F (**10**)	1.0	2.7	3.0
F (**11**)	2.8	2.0	13.5
G (**12**)	3.2	30.0	30.0
Doxorubicin	0.2	0.0055	0.5

[a]ID_{50} values were determined on day 3 culture.

[b]n.d. = Not determined.

Vismione A (**1**), the most effective compound against 9 KB cells in vitro, was tested in vivo against some experimental tumors. The data, obtained under the auspices of the NCI, Division of Cancer Treatment, Drug Research and Development Branch, and reported in Tables 2 and 3, show that in mice vis-

TABLE 2. Antitumor Activity of Vismione A against M 5076 Ovarian Carcinoma Transplanted ip[a] (Data from National Cancer Institute)

Vismione A	mg/kg	T/C (%)
(NSC 339659)	0.31	111
	0.63	115,108
	1.25	119,111
	2.50	140,135
	5.0	145,147
	10.0	toxic

[a]Male BDF_1 mice transplanted ip with 1/10 dilution of M 5076; treatment ip from day 1 to 9 after transplantation. Vismione A was administered as a solution/suspension in Klucel (hydroxypropylcellulose).

[1]G. Cassinelli and A. Sanfilippo, unpublished results.

TABLE 3. Antitumor Activity of Vismione A against B16 Melanocarcinoma Transplanted ip[a] (Data from National Cancer Institute)

Vismione A	mg/kg	T/C(%)
(NSC 339659)	0.62	98
	1.25	112
	2.50	115
	5.0	148
	10.0	toxic

[a]Male BDF_1 mice transplanted ip with 1/10 dilution of B16; treatment ip from day 1 to 5 after transplantation. Vismione A was administered as a solution/suspension in Klucel (hydroxypropylcellulose).

mione A displays a significant activity against M 5076 ovarian carcinoma and B16 melanocarcinoma; it is inactive against P-388 ascitic leukemia (T/C 117% at 5 mg/kg).

EXPERIMENTAL

PLANT MATERIAL.—Vismiones A (**1**) and B (**4**) were isolated from the fruits of *Vismia baccifera* var. *dealbata* (H.B.K.) Ewan (11); deacetylvismione A (**2**) from the fruits of *Vismia lindeniama* Dcne. (12); acetylvismione B (**3**) from the fruits of *Vismia japurensis* Reich. (13); vismione C (**5**), D (**8**), and E (**6**) from the fruits of *Psorospermum febrifugum* Spach (14); vismione H (**9**) and acetylvismione D (**7**) from the roots of *Psorospermum tenuifolium* Hook. (15); acetylvismione F (**10**), vismione F (**11**) and G (**12**) from the roots of *Psorospermum corymbiferum* Hochr. and *Psorospermum glaberrimum* Hochr. (16).

BIOLOGICAL EVALUATION.—Before testing, samples of ethanolic solutions of vismiones were diluted with distilled H_2O to obtain a final EtOH concentration of 0.2%.

P-388 leukemia cells resistant to doxorubicin (P-388/R) were obtained by F.M. Schabel of Southern Research Institute, Birmingham, Alabama, after repeated exposure of P-388 cells to doxorubicin.

The cytotoxicity on the different cell lines was determined by counting surviving cells after 72 h of continuous exposure to the drugs (17). The percentage of controls and the ID_{50} (inhibiting dose) values were calculated on dose-response curves.

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