Structure Activity Relationships of Antiproliferative Rocaglamide Derivatives from *Aglaia* Species (Meliaceae)

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Eleven rocaglamide derivatives (cyclopentatetrahydrobenzofurans) and one structurally related aglain congener all isolated from different *Aglaia* species (Meliaceae) were tested for growth inhibiting properties using the human cancer cell lines MONO-MAC-6 and MEL-JUSO. Proliferation of both cell lines was efficiently inhibited in a dose and compound dependent manner. Applying a MTT-Assay, the IC_{50} of the most active compound didesmethylrocaglamide **(1)** was observed at 0.002 and 0.006 μg/ml (0.004 and 0.013 μM) depending on the cell line investigated. Bulky aminoacyl substituents at C-2, acetylation of the OH substituent at C-1 or insertion of a OH or OMe substituent at C-3' of the rocaglamide skeleton all diminished the activity of the compounds investigated. The aglain derivative **12** was inactive up to a concentration of 3 μg/ml (4.6 μM). This loss of activity is assumed to be mainly due to the presence of a pyran ring in the aglains vs. a furan ring as found in rocaglamide derivatives. Rocaglamide derivatives may act primarily by inhibition of cell proliferation as evidenced by the absence of a significant cytotoxic effect in long-term cultures of MONO-MAC-6 cells treated with high doses of didesmethylrocaglamide.

Our data suggest that rocaglamide derivatives could exert a potential role in the treatment of malignant diseases and are worth to be investigated in further studies of experimental medicine and pharmacology.

Introduction

The genus *Aglaia* (Meliaceae) consists of approximately 130 species that are dioecious trees or shrubs with small fragrant flowers (Li, 1977; Ridley, 1922). Species of *Aglaia* form an important element of moist tropical forests in the Indo-Malaysian region (Pannel, 1992). Several species, such as *A. odorata*, are traditionally used in folk medicine in South-East Asia for example as a heart stimulant, as febrifuge, for the treatment of coughs, inflammations or injuries (Kokpol *et al.*, 1994). Other *Aglaia* species including *A. duperreana* are widely cultivated in Vietnam, China and in parts of Indo-Malaysia for their fragrant flowers which are used for scenting tea.

Phytochemically *Aglaia* species are characterized by the accumulation of cyclopentatetrahydrobenzofurans **(1)** also known as rocaglamide derivatives (Nugroho *et al.*, 1997a; Nugroho *et al.*, 1997b; Güssregen *et al.*, 1997; Dumontet *et al.*, 1996; Ewete *et al.*, 1996; Ishibashi *et al.*, 1993; Janprasert *et al.*, 1993; King *et al.*,1982). Rocaglamide derivatives are powerful natural insecticides which are comparable, with regard to their insecticidal activity, to the well known natural insecticide

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azadirachtin isolated from the neem tree *Azadirachta indica* (Nugroho *et al.*, 1997a; Ewete *et al.*, 1996). Recently, a pronounced antiproliferative activity of certain rocaglamide derivatives against human cancer cells was reported (Cui *et al.*, 1997; Wu *et al.*, 1997) prompting us to conduct a comparative study on the cytotoxic activity of eleven naturally occurring rocaglamide derivatives **1–11** and of one closely related aglain derivative **12** using two human cancer cell lines derived from monocytic leukemia and melanoma. Here we report on the structure-activity relations of antiproliferative rocaglamide derivatives.

Materials and Methods

Plant material

Flowers and stems of *Aglaia duperreana* were collected in Vietnam (near Ho Chi Minh City) in April 1995 and January 1998 and were specified by one of us (L. C. K., Dept. of Botany, University HCMC). Flowers of *Aglaia odorata* and seeds of *Aglaia elliptica* were supplied in December 1994 by the Botanic Garden at Bogor, Indonesia. Voucher specimens are on file in the J.-v.-Sachs-Institut für Biowissenschaften, Universität Würzburg.

Isolation of the rocaglamide derivatives

The isolation and structural elucidation of the rocaglamide derivatives with complete data of MS, ^{1}H- and ^{13}C-, 2D-NMR, CD and optical rotation were previously described (Nugroho *et al.*, 1997a; Nugroho *et al.*, 1997b; Güssregen *et al.*, 1997). Detailed spectroscopic data of the aglain derivative **12** are published separately elsewhere (Nugroho, 1998).

Chemicals

MTT [3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazoliumbromide] was purchased from Sigma (Deisenhofen, Germany). RPMI 1640-Cell culture medium and supplements, fetal bovine serum (FBS), penicillin and streptomycin were obtained from Gibco-BRL (Eggenstein, Germany). EGMME (ethylene glycol monomethyl ether) was from Merck (Darmstadt, Germany).

Proliferation assays

The rocaglamide derivatives and the aglain derivative were tested for their cytotoxic activity against the two human cancer cell lines MONO-MAC-6 (Ziegler-Heitbrock *et al.*, 1988) (human acute monocytic leukemia) and MEL-JUSO (Ziegler-Heitbrock *et al.*, 1985; Johnson *et al.*, 1981) (human melanoma). The cell lines are deposited at the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany).

The cells were grown in plastic flasks (Nunc, Wiesbaden, Germany). All cultures were propagated using standardized media supplemented with 10–20% heat-inactivated fetal bovine serum and were cultivated at 37 °C in a humidified atmosphere containing 5% CO_2. Details are outlined in the DSMZ Catalogue of Human and Animal Cell Lines (Drexler *et al.*, 1997).

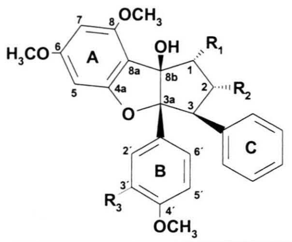
The freeze-dried rocaglamides were dissolved in EGMME (ethylene glycol monomethyl ether), diluted in RPMI-1640 culture medium and stored frozen at −20 °C. At the final dilution used in the experiments, the EGMME concentration was 0.1%.

For the experiments, exponentially growing cells (viability of >90% as measured by trypan blue exclusion) were harvested, washed and resuspended in fresh medium at a final concentration ranging between 2 and 4 x 10^5 cells/ml (depending on the cell line). Total cell number and viability were determined in a cell counting chamber after staining the cells with trypan blue. Aliquots of 90 µl were seeded out into 96-well flat-bottom culture plates (Nunc, Wiesbaden, Germany). Ten microliters with medium, medium with the solvent EGMME or the different concentrations of the rocaglamide derivatives were added. After a 48 h incubation/cultivation period the extent of cytotoxicity was evaluated by the MTT assay as described recently (Steube *et al.*, 1998).

All experiments were carried out in triplicates and repeated three times. As controls, media with 0.1% EGMME were included in the experiments. To avoid bacterial contamination all experiments were carried out in the presence of 100 IU/ml penicillin G and 100 µg/ml streptomycin.

Results and Discussion

The antiproliferative activity of rocaglamide derivatives **1–11** as well as of the structurally related aglain derivative **12** was investigated using two human cancer cell lines MONO-MAC-6 and MEL-JUSO that are firmly established in anti-cancer research. MONO-MAC-6 grows as suspension culture and is derived from a monocytic leukemia representing typical features of monocytic cells (Ziegler-Heitbrock *et al.*, 1988) and is widely used as model systems in hematology and immunology (Steube and Drexler, 1995) and MEL-JUSO which grows in monolayers was established from the solid tumor mass of a melanoma (Johnson *et al.*, 1981).



Compound	R$_1$	R$_2$	R$_3$
1	OH	CONH$_2$	H
2	OH	CONHCH$_3$	H
3	OH	CON(CH$_3$)$_2$	H
4	OH	CONH(CH$_2$)$_4$OH	H
5	OH	CONH$_2$	OH
6	OH	CONHCH$_3$	OH
7	OH	CON(CH$_3$)$_2$	OH
8	OCOCH$_3$	CON(CH$_3$)$_2$	OH
9	OH	CON(CH$_3$)$_2$	OCH$_3$
10	OH	COOCH$_3$	OH
11	OCOCH$_3$	CONHCH$_3$	OH

Fig. 1. Chemical structures of the1*H*-cyclopentatetrahydrobenzofuran derivatives (rocaglamide derivatives) **1–11**.

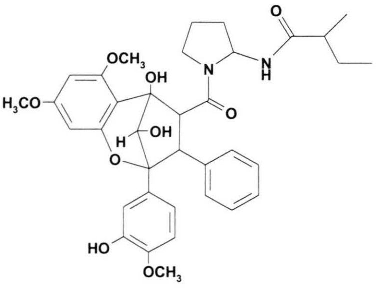


Fig. 2. Chemical structure of the cyclopentatetrahydrobenzopyran derivative (aglain derivative) **12**.

The rationale for studying a series of rocaglamide derivatives **1–11** for their antiproliferative effect was based on the known structural diversity of naturally occurring rocaglamides that usually differ from each other by the nature of their substituents at C-1 (-OH vs. -OAC), C-2 (different amino acyl substituents vs. -COOCH$_3$ group) and/or C-3' (-H vs. -OH or -OCH$_3$) whereas the substitution pattern at the remaining carbons of the rocaglamide skeleton as well as the absolute configuration of the various congeners are remarkably stable (Nugroho *et al.*, 1997a; Nugroho *et al.*, 1997b; Güssregen *et al.*, 1997). Only didesmethylrocaglamide **(1)** and rocaglamide **(3)** had been tested earlier (Dumontet *et al.*, 1996; Wu *et al.*, 1997) whereas the cytotoxic activity of the remaining compounds is reported here for the first time. The aglain derivative **12** that sometimes cooccurs together with rocaglamide-type compounds, as for example in *A. odorata* (Nugroho, 1998), was included into the bioassays since it is structurally related to rocaglamide **(3)** and its congeners but differs from the latter by the nature of its heterocycle which is a pyran ring in **12** compared to a furan ring in **1–11**, as well as by the size of the aminoacyl substituent.

All of the compounds studied except the aglain derivative **12** exhibited pronounced antiproliferative activity against the two cell lines in the MTT-assay (Table I). The IC$_{50}$ of didesmethylrocaglamide **(1)** which turned out to be the most active compound detected in this study was 0.002 and 0.006 µg/ml (corresponding to 0.004 and 0.013 µM) for MONO-MAC-6 and MEL-JUSO, respectively and is thus comparable to that of the wellknown anticancer drug vinblastine sulfate (Table I). Replacement of the CONH$_2$ substituent at C-2 by a larger aminoacyl substituent as in compounds **3** or **4** caused a slight but noticeable and reproducible decrease in inhibitory activity (between two- to eightfold) compared to didesmethylrocaglamide **(1)**.

A further decrease of antiproliferative activity was observed when C-3' was substituted by an OH-group as in compounds **5–7**. For example, whereas the IC$_{50}$ of didesmethylrocaglamide **(1)** was 0.002 µg/ml (0.004 µM) for MONO-MAC-6 cells this value increased to 0.018 µg/ml (0.037 µM)

Table I. IC_{50}-values of compounds **1–12** determined for two human cancer cell lines[a].

	Cell line[b]			
Compound	MONO-MAC-6 [µg/ml]	[µM]	MEL-JUSO [µg/ml]	[µM]
1	0.002	(0.004)	0.006	(0.013)
2	0.011	(0.022)	0.015	(0.031)
3	0.016	(0.032)	0.009	(0.018)
4	0.013	(0.024)	0.012	(0.022)
5	0.018	(0.037)	0.025	(0.051)
6	0.053	(0.105)	0.041	(0.081)
7	0.028	(0.054)	0.021	(0.040)
8	0.046	(0.082)	0.032	(0.057)
9	0.092	(0.172)	0.049	(0.092)
10	0.123	(0.242)	0.070	(0.138)
11	0.103	(0.188)	0.090	(0.164)
12	N.A[c]		N.A[c]	

The IC_{50} values reported represent means of three independent experiments.

[a] Results are expressed as IC_{50} values in µg/ml (µM). Values for standard antitumor drug towards MONO-MAC-6 were: vinblastine sulfate 0.0085 (0.009).

[b] Key: MONO-MAC-6 = human acute monocytic leukemia cell line.
MEL-JUSO = human melanoma cell line.

[c] Not active up to 3 µg/ml (4.6 µM).

for compound **5** which is the 3'-OH analogue of **1**. A similar result was obtained when the compounds were tested in MEL-JUSO cells (Table I).

Acetylation of the OH-substituent at C-1 as in compounds **8** and **11** resulted in an approximately twofold loss of activity as compared to the corresponding deacetyl congeners **6** and **7**: the IC_{50} of compound **6** for MONO-MAC-6 cells was 0.053 µg/ml (0.105 µM), the IC_{50} of the acetylated congener **11** amounted to merely 0.103 µg/ml (0.188 µM). A similar trend with regard to the bioactivity of acetylated vs. deacetylated rocaglamide derivatives was observed when testing MEL-JUSO cells.

Replacement of the amino acyl substituent of the rocaglamide derivatives **5–7** by an esterified acyl group as in compound **10** resulted in a decrease of the antiproliferative activity in both cell lines tested as evident from inspection of the IC_{50} values of compounds **5–7** compared to **10** (Table I).

The aglain derivative **12** which differs from the rocaglamide congeners **1–11** by presence of a pyran vs. a furan ring and by a larger aminoacyl side chain was completely inactive in both cell lines even when tested at concentrations as high as 3 µg/ml (4.6 µM) which is roughly 20–30 times higher than the IC_{50} values of the weakest rocaglamide derivatives **10** or **11** encountered during this study (Table I). It may be assumed that the dramatic loss of activity of **12** observed in this study is rather due to the replacement of the furan ring of the rocaglamide derivatives by the pyran ring of the aglain derivative **12** than by the bulky aminoacyl substituent of **12**. The size of the amino acyl side chain at C-2 has a small but significant influence on the degree of antiproliferative activity of the various rocaglamide derivatives investigated as evidenced by the IC_{50} values of compounds **1–4**, implying a small substituent as beneficial for antiproliferative activity. However, a total loss of activity as experienced for compound **12** is probably not attributed to this structural feature alone. Applying a second proliferation assay, the incorporation of [^{3}H]-thymidine for several selected rocaglamide derivatives, confirmed the results obtained by the MTT-test (data not shown).

It is interesting to note that the structure activity relationships of compounds **1–12** analyzed in this study (Table I) are largely paralleled by their different degree of insecticidal activity which was reported earlier using larvae of the polyphagous pest insect *Spodoptera littoralis* (Nugroho, 1998; Nugroho *et al.*, 1997a; Nugroho *et al.*, 1997b; Güssregen *et al.*, 1997). It is therefore most likely that the hitherto unknown target(s) of rocaglamide derivatives in human cancer cells are similar or identical to the targets present in insects.

In a further set of experiments the survival-rate of MONO-MAC-6 cells was determined after a 48 h exposure to different concentrations of didesmethylrocaglamide **(1)**, which represents the most active compound encountered in this study (Fig. 3). After an initial exposure of the cells to didesmethylrocaglamide **(1)** for 48 h the cells were washed and transferred to control medium (with 0.1% EGMME but without compound **(1)**) for subsequent 48 h. The concentrations of compound **1** applied for this experiment (0.020, 0.050 and 0.100 µg/ml) were by far beyond the determined IC_{50} value of 0.002 µg/ml. Even at these exceedingly high concentrations only an insignificant number of cells died during the first 48 h of the experiment as indicated by a constant number of viable cells (Fig. 3). Following transfer into fresh medium without compound **1** the number of viable cells in the culture, which had been treated with

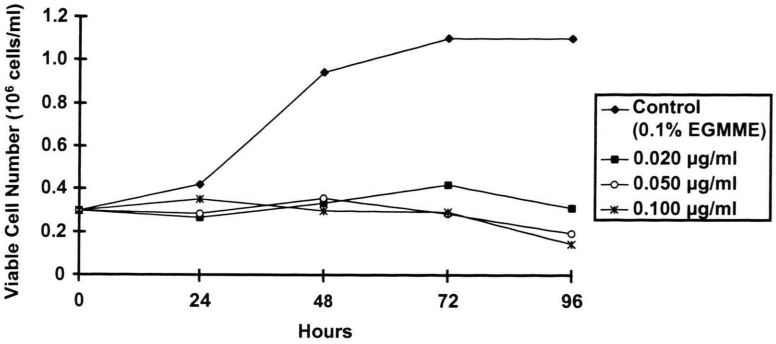


Fig. 3. Effect of didesmethylrocaglamide **(1)** on the number of viable MONO-MAC-6 cells. The cells were incubated in the presence of **1** at three different concentrations (0.020, 0.050 and 0.100 µg/ml), after 48 hours cells were washed and further incubated without the drug for additional 48 hours. Numbers of viable cells were determined in a cell counting chamber after staining the cells with trypan blue dye. As control serves a culture incubated with 0.1% EGMME (ethylene glycol monomethyl ether), since the drugs were dissolved in a final conc. of 0.1% EGMME.

the lowest concentration of **1** (0.020 µg/ml) remained almost constant whereas the viable cell numbers of the cultures which had been pretreated with 0.050 or 0.100 µg/ml of compound **1** decreased slightly (Fig. 3). This experiment indicated that didesmethylrocaglamide **(1)** (and probably also the other rocaglamide derivatives) clearly inhibits cell proliferation but is only sligthly toxic to MONO-MAC-6 cells even at concentrations that are 25–50 times higher than its IC_{50} of **1** as determined by the MTT assay (Table I). The experiment indicated furthermore that the antiproliferative effect of compound **1** can not easily be neutralized by transferring the exposed MONO-MAC-6 cells into fresh medium since the cell numbers did not increase from 48–96 h following onset of the experiment (Fig. 3). It is possible that compound **1** which is rather lipophilic, enters the cells, binds to an unknown target (thereby suppressing cell proliferation) and could not be liberated from its intracellular target by washing the treated cells and subsequent culture in normal medium. Since these compounds may be interesting candidates for anti-cancer research, further research on the mode of action of antiproliferative rocaglamide derivatives is required.

In Vietnam as well as in other South East Asian countries the fragrant flowers of *A. duperreana* or of *A. odorata* are commonly added to tea thereby making a popular drink that is consumed daily by large parts of the population on a regular basis (Perry and Metzger, 1980). Flowers from both *Aglaia* species are known to contain rocaglamide derivatives. Even though these compounds are rather lipophilic in nature they are extractable in hot water and can be detected in a typical herbal tea using HPLC analysis (Bohnenstengel *et al.,* unpublished results). We treated three purified rocaglamide derivatives, compounds **1–3**, with boiling water and proved that these compounds are stable and not subject to heat-induced chemical alterations. Even though no effort was made so far to determine the actual concentrations of rocaglamide derivatives present in a typical tea scented with *Aglaia* flowers it is conceivable that trace amounts of these compounds are incorporated on a regular basis over many years. The fate of ingested rocaglamide derivatives in humans (e.g. resorption, pharmacokinetics, metabolism) is completely unknown. Due to the potential antiproliferative activity of rocaglamide derivatives and in view of the regular and prolonged exposure of a considerable part of the South East Asian population to rocaglamides, these compounds represent interesting subjects for further studies.

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