**Title:** NEW USE OF CITREAMICINS

**Abstract:**
Citreamicins possess useful antitumor activity. Typically they are of formula (I), wherein \(R_1\) is selected from the group consisting of \(\text{COCH}_2\text{CH(CH}_3)_2\text{, COCH(CH}_3)_2\text{, COCH}_3\) or \(H\) when \(R_2\) is \(\text{CH}_3\), or wherein \(R_1\) is \(\text{COCH}_2\text{CH(CH}_3)_2\) and \(R_2\) is \(H\).
NEW USE OF CITREAMICINS

Citreamicins possess useful antitumor activity. Typically they are of formula (I), wherein R₁ is selected from the group consisting of COCH₁CH(CH₃)₂, COCH(CH₃)₂, COCH₁ or H when R₂ is CH₃, or wherein R₁ is COCH₂CH(CH₃)₂ and R₂ is H.

\[ \text{(I)} \]

[Continued on next page]
MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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NEW USE OF CITREMICINS

The present invention relates to citreomicins, and in particular to a new use of the citreomicins.

BACKGROUND

The citreomicins are known compounds, see: Pearce, C.J.; Carter, G.T.; Nietsche, J.A.; Borders, D.B.; Greenstein, M. and Maiese, W.M. J. Antibiotics, 1991, 44(11), 1247-1250. The citreomicins thus include compounds of the formula:

![Citreomicin Structure](image)

wherein $R_1$ is selected from the group consisting of $\text{COCH}_2\text{CH(CH}_3)_2$, $\text{COCH(CH}_3)_2$, $\text{COCH}_3$ or $\text{H}$ when $R_2$ is $\text{CH}_3$, or wherein $R_1$ is $\text{COCH}_2\text{CH(CH}_3)_2$ and $R_2$ is $\text{H}$. In particular, citreamicin $\alpha$ is a compound of formula (I) where $R_1$ is $\text{COCH}_2\text{CH(CH}_3)_2$ and $R_2$ is $\text{CH}_3$.  

SUMMARY OF INVENTION
We have now found a new use of the known citreamicins, especially those of
the formula (I). We have found that they exhibit antitumor activity.

Thus, we provide pharmaceutical compositions for treatment of tumors and
which include a citreamicin and a pharmaceutically acceptable carrier.

We further provide methods of making such pharmaceutical compositions,
including the use of a citreamicin in the preparation of a medicament for use in
treating a tumor.

Additionally, we provide a method for treating a mammal affected by a
malignant tumor sensitive to a citreamicin compound such as a compound of formula
(I), which comprises administering to the affected individual a therapeutically
effective amount of the citreamicin compound or a pharmaceutical composition
thereof.

DETAILS OF THE INVENTION

Examples of pharmaceutical compositions include any solid (tablets, pills,
capsules, granules, etc.) or liquid (solutions, suspensions or emulsions) with suitable
formulation of oral, topical or parenteral administration, and they may contain the
pure compound or in combination with any carrier or other pharmacologically active
compounds. These compositions may need to be sterile when administered
parenterally.

The correct dosage of a pharmaceutical composition comprising a citreamaicin
compound will vary according to the pharmaceutical formulation, the mode of
application, and the particular situs, host and tumor being treated. Other factors like
age, body weight, sex, diet, time of administration, rate of excretion, condition of the
host, drug combinations, reaction sensitivities and severity of the disease shall be
taken into account. Administration can be carried out continuously or periodically
within the maximum tolerated dose.
We have found in particular that citreamicin α exhibits in vitro antitumor activity against a cell line derived from mouse lymphoma.

**BILOGICAL ACTIVITY**

Citreamicin α displays good antitumor activity. Its antitumor activity has been detected in vitro by culturing the tumor cells following the methodology described by


Cells were maintained in logarithmic phase of growth in Eagle's Minimum Essential Medium, with Earle's Balanced Salts, with 2.0 mM L-glutamine, with non-essential amino acids, without sodium bicarbonate (EMEM/NEAA); supplemented with 10% Fetal Calf Serum (FCS), 10⁻² M sodium bicarbonate and 0.1 g/l penicillin-G + streptomycin sulfate.

A screening procedure has been carried out to determine and compare the antitumor activity of citreamicin CL, using an adapted form of the method described by Bergeron et al. The antitumor cells employed were P388 (ATCC CCL-46, suspension culture of a lymphoid neoplasm from DBA/2 mouse), A549 (ATCC CCL-185, monolayer culture of a human lung carcinoma) and HT-29 (ATCC HTB-38, monolayer culture of a human colon carcinoma).

P388 cells were seeded into 16 mm wells at 1 x 10⁴ cells per well in 1 ml aliquots of MEM 5FCS containing the indicated concentration of drug. A separate set of cultures without drug was seeded as control growth to ensure that cells
remained in exponential phase of growth. All determinations were carried out in
duplicate. After three days of incubation at 37°C, 10% CO₂ in a 98% humid
atmosphere, an approximate IC₅₀ was determined by comparing the growth in wells
with drug to the growth in wells control.

A₅₄⁹ and HT-29 cells were seeded into 16 mm wells at 2 x 10⁴ cells per well in
1 ml aliquots of MEM 10FCS containing the indicated concentration of drug. A
separate set of cultures without drug was seeded as control growth to ensure that cells
remained in exponential phase of growth. All determinations were carried out in
duplicate. After three days of incubation at 37°C, 10% CO₂ in a 98% humid
atmosphere, the wells were stained with 0.1 % Crystal Violet. An approximate IC₅₀
was determined by comparing the growth in wells with drug to the growth in wells
control.

The activity results, IC₅₀ (µM), for citreamicin α are given in the following
table:

<table>
<thead>
<tr>
<th></th>
<th>P₃₈₈</th>
<th>A₅₄⁹</th>
<th>HT-29</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC CCL-46</td>
<td>0.003</td>
<td>0.004</td>
<td>0.004</td>
</tr>
<tr>
<td>ATCC CCL-185</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATCC HTB-38</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citreamicin α</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Claims


2. The use according to claim 1, wherein the citreamicin is of the formula (I),

\[
\begin{align*}
&\text{OR}_1 \\
&\text{CH}_3 \\
&\text{O} \\
&\text{N} \\
&\text{CH}_3 \\
&\text{OH} \\
&\text{O} \\
&\text{COOH} \\
&\text{OR}_2 \\
&\text{OCH}_3
\end{align*}
\]

wherein \( R_1 \) is selected from the group consisting of \( \text{COCH}_2\text{CH(CH}_3)_2 \), \( \text{COCH}(\text{CH}_3)_2 \), \( \text{COCH}_3 \) or \( H \) when \( R_2 \) is \( \text{CH}_3 \), or wherein \( R_1 \) is \( \text{COCH}_2\text{CH(CH}_3)_2 \) and \( R_2 \) is \( H \).

3. The use according to claim 2, wherein the citreamicin is citruscin \( \alpha \) which is of formula (I) where \( R_1 \) is \( \text{COCH}_2\text{CH(CH}_3)_2 \) and \( R_2 \) is \( \text{CH}_3 \).

4. A method of treating a tumor which comprises administration of an effective
amount of a citreamicin compound.

5. A method according to claim 4, wherein the citreamicin is of the formula (I),

6. A method according to claim 5, wherein the citreamicin is citreamicin α which is of formula (I) where \( R_1 \) is \( \text{COCH}_2\text{CH(CH}_3)_2 \), \( \text{COCH}_2\text{OH} \) or \( \text{H} \) when \( R_2 \) is \( \text{CH}_3 \), or wherein \( R_1 \) is \( \text{COCH}_2\text{CH(CH}_3)_2 \) and \( R_2 \) is \( \text{H} \).

7. A pharmaceutical composition with antitumor activity comprising a citreamicin and a pharmaceutically acceptable carrier.

8. A pharmaceutical composition according to claim 7, wherein the citreamicin is of the formula (I),
wherein R₁ is selected from the group consisting of COCH₂CH(CH₃)₂, COCH(CH₃)₂, COCH₃ or H when R₂ is CH₃, or wherein R₁ is COCH₂CH(CH₃)₂ and R₂ is H.

9. A pharmaceutical composition according to claim 8, wherein the citreamicin is citreamicin α which is of formula (I) where R₁ is COCH₂CH(CH₃)₂ and R₂ is CH₃.