Synthesis, Physicochemical Characterization and Study of the Antimicrobial Activity of Chlorobutanol

H. Nadia, G. Bahdja, S. Thili Malha, Y. Zahoua, D. Taoufik, B. Mourad, M. Marzouk, F. Z. Hadjadj Aoul, L. R. Mekacher

Abstract—Introduction and objectives: Chlorobutanol is a raw material, mainly used as an antiseptic and antimicrobial preservative in injectable and ophthalmic preparations. The main objective of our study was the synthesis and evaluation of the antimicrobial activity of chlorobutanol hemihydrates. Material and methods: Chlorobutanol was synthesized according to the nucleophilic addition reaction of chloroform to acetone, identified by an infrared absorption using Spectrum One FTIR spectrometer, melting point, Scanning electron microscopy and colorimetric reactions. The dosage of Carvedilol active substance was carried out by assaying the degradation products of chlorobutanol in a basic solution. The chlorobutanol obtained was subjected to bacteriological tests in order to study its antimicrobial activity. The antibacterial activity was evaluated against strains such as Escherichia coli (ATCC 25 922), Staphylococcus aureus (ATCC 25 923) and Pseudomonas aeruginosa (ATCC = American type culture collection). The antifungal activity was evaluated against human pathogenic fungal strains, such as Candida albicans and Aspergillus niger provided by the parasitology laboratory of the Hospital of Tizi-Ouzou, Algeria. Results and discussion: Chlorobutanol was obtained in an acceptable yield. The characterization tests of the product obtained showed a white and crystalline appearance (confirmed by scanning electron microscopy), solubilities (in water, ethanol and glycerol), and a melting temperature in accordance with the requirements of the European Pharmacopoeia. The colorimetric reactions were directed towards the presence of a trihalogenated carbon and an alcohol function. The spectral identification (IR) showed the presence of characteristic chlorobutanol peaks and confirmed the structure of the latter. The microbiological study revealed an antimicrobial effect on all strains tested (Staphylococcus aureus (MIC = 1250 µg/ml), E. coli (MIC = 1250 µg/ml), Pseudomonas aeroginosa (MIC = 1250 µg/ml), Candida albicans (MIC =2500 µg/ml), Aspergillus niger (MIC =2500 µg/ml)) with MIC values close to literature data. Conclusion: Thus, on the whole, the synthesized chlorobutanol satisfied the requirements of the European Pharmacopoeia, and possesses antibacterial and antifungal activity; nevertheless it is necessary to insist on the purification step of the product in order to eliminate the maximum impurities.

Keywords—Antimicrobial agent, bacterial and fungal strains, chlorobutanol, MIC.
• Milieu Chapman for *Staphylococcus aureus*
• Milieu Hektoen for *Pseudomonas aeruginosa* and *E. coli*.

### TABLE I

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Strains tested</th>
<th>Species tested</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>bacteria</td>
<td>Gram -</td>
<td><em>Escherichia coli</em> (ATCC 27 853)</td>
<td>Reference strains American type culture collection (ATCC)</td>
</tr>
<tr>
<td></td>
<td>Gram +</td>
<td><em>Pseudomonas aeruginosa</em> (ATCC 922)</td>
<td></td>
</tr>
<tr>
<td>fungal species</td>
<td></td>
<td><em>Aspergillus niger</em></td>
<td>Parastology laboratory of Tizi-Ouzou hospital</td>
</tr>
</tbody>
</table>

### Table II

<table>
<thead>
<tr>
<th>DILUTION RANGE OF CHLOROBUTANOL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution ratio</td>
</tr>
<tr>
<td>[ClbH] %</td>
</tr>
<tr>
<td>[ClbH] in μg/ml</td>
</tr>
</tbody>
</table>

*ClbH*: concentration of chlorobutanol hemihydrate.

### Preparation of the Dilution Range of Synthesized Chlorobutanol

#### a. Preparation of the Stock Solution at 5000 μg/ml (0.5%)

We weigh with an analytical balance 58.82 mg of chlorobutanol hemihydrate synthesized which corresponds to 50 mg of pure chlorobutanol (test portion corrected for the content of active ingredient which is 85%), solubilized with 50 mg of pure chlorobutanol (test portion corrected for the chlorobutanol hemihydrate synthesized which corresponds to 1.108 CFU/ml. In our work, in the absence of densitometer, we have estimated this density to 1 or 2 identical and well isolated colonies which would cause a slight disturbance observed with the naked eye under the white light of the day.

#### b. Preparation of Dilutions

- We perform semi-logarithmic dilutions of half to half of the chlorobutanol hemihydrate synthesized for a concentration range from 5000 μg/ml up to 312.5 μg/ml (Table II).
- We prepare a dilution range for each strain tested, i.e. *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, *Aspergillus niger*.
- We provide negative control tubes for each dilution series (without microbial inoculum).
- *Preparation of the Microbial Inoculums*: The microbial stock suspension should have a turbidity of 0.5 MacFarland which corresponds to 1.108 CFU/ml. In our work, in the absence of densitometer, we have estimated this density to 1 or 2 identical and well isolated colonies which would cause a slight disturbance observed with the naked eye under the white light of the day.

#### c. Preparation of the Bacterial Suspension

- From a young culture of 24 hours and using a platinum loop, we take one to two bacterial colonies well isolated and identical;
- We introduce the collected colonies in about 5 to 10 ml sterile physiological saline 0.9% NaCl, we homogenize vortex and check for the appearance of a slight disorder;
- We take 100 μl of the bacterial inoculum and introduce it into 9.9 ml of sterile physiological saline to obtain a 1/100 dilution.

#### d. Preparation of the Fungal Suspension

- Using a sterile loop, we take 1 to 2 fungal colonies;
- We Immerse the collected colonies in about 5 to 10 ml sterile physiological saline, we homogenize and check for the appearance of a slight disorder;
- We take 100 μl of the fungal inoculum and introduce it into 9.9 ml of sterile physiological saline;

#### e. Distribution of the Microbial Inoculums

- The distribution of the inoculum must be done within 15 minutes of its preparation. We incorporate 100 μl of microbial suspension into each tube of the dilution range;
- We proceed from the lowest concentration to the highest concentration;
- We prepare a positive control tube; by incorporating 100 μl of the inoculum into a tube containing 5 ml of BGT without chlorobutanol (Fig. 2).
- We prepare a control box (control box) by spreading with a rake 100 μl of each suspension on a nutrient agar for bacteria and Sabouraud medium without antibiotic for yeast and mold, this control allows us to check the purity of each strain and estimate the microbial density (Fig. 3).

#### f. Incubation

- We incubate the broths and the controls, in incubators programmed according to the conditions mentioned in Table III.

#### g. Reading the MIC

- The reading of the MIC is done visually, by observing the presence or absence of turbidity in incubated broth;
- The MIC value is the lowest concentration of the antimicrobial that inhibits any visible bacterial growth with the naked eye, which is the concentration of the first clear tube in the dilution range.
TABLE III
INCUBATION CONDITIONS OF THE MICROORGANISMS TESTED

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Incubation temperature</th>
<th>Incubation time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>37 °C</td>
<td>24 hours</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>37°C</td>
<td>24 hours</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>37°C</td>
<td>24 hours</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>37°C</td>
<td>48 hours</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>25°C</td>
<td>48 hours</td>
</tr>
</tbody>
</table>

- The MIC value characterizes the bacteriostatic and fungistatic effect of an antimicrobial [4].

III. RESULTS AND DISCUSSION

A. Chemistry

Chlorobutanol is obtained with a yield of 54%, it is in the form of white crystalline powder with a very strong camphor flavor (Figs. 4 and 5), slightly soluble in water, freely soluble in ethanol at 96 percent and soluble in glycerol at 85 percent. The melting point obtained with the synthesized chlorobutanol is 78.0 °C.
The reaction of pyridine with our product gave a red layer on the surface after heating. This red layer corresponds to the Fujiwara-Ross reaction which directs towards the trichlorinated carbon of chlorobutanol hemihydrate.

The reaction of chlorobutanol with ammoniacal silver nitrate gave a black precipitate. This precipitate corresponds to the silver hydroxide obtained in alkaline medium from AgCl; product of the reaction between chloride ions (released after degradation of chlorobutanol) with silver nitrate (Fig. 7).

The reaction of chlorobutanol with potassium iodide gave a yellowish precipitate. This precipitate corresponds to the formation of iodoform from potassium iodide and acetone resulting from the degradation of the alcohol function of chlorobutanol in alkaline medium (Fig. 7).

Absorption spectra in IR of synthesized chlorobutanol: The spectra absorption obtained with synthesized chlorobutanol is shown in Fig. 8.

To interpret the infrared absorption spectrum of synthesized chlorobutanol, we divide it into two main regions:

- The functional group region of 4000 cm⁻¹ to 1500 cm⁻¹:
  - The bands 1459.26 cm⁻¹; 1440.87 cm⁻¹; 1385.39 cm⁻¹ and 917.83 cm⁻¹ due to different deformations of the C-H bonds of the methyls;
  - We have been able to confirm with the bands 1370.33 cm⁻¹, 983.16 cm⁻¹, 565.17 cm⁻¹, 442.3 cm⁻¹ the presence of a tertiary alcohol bonded to an alkane, substituted by methyl radicals in the structure of our product.
  - The bands 791.18 cm⁻¹ and 612.69 cm⁻¹ confirm the presence of a trichlorinated carbon;
  - In addition, the 833.30 cm⁻¹-band confirms that tertiary alcohol and trichlorinated carbon belong to the same compound.
  - However, the presence of two strands foreign to the structure of chlorobutanol, namely the 704.05 cm⁻¹ and the 1651.74 cm⁻¹ band, which point to a monosubstituted benzyl, is probably due to the presence of the related chlorobutanol substances, namely phenoxyethanol or phenylethanol.

B. Antimicrobial Activity

The microbiological study revealed an antimicrobial effect on all strains tested, with MIC values close to literature data.

For Escherichia coli, we observed a disorder in the positive control tube, the 0.031% dilution and the 0.062% dilution indicating the presence of a bacterial outbreak. However, no disturbances were observed in the negative control tube, the 0.125% dilution, the 0.25% dilution and the 0.5% dilution.
The value of the MIC of the chlorobutanol hemihydrate synthesized on the *Escherichia coli* strain (ATCC 25 922) tested is therefore 0.125%:

$$\text{MIC}_{\text{Chlorobutanol-}E.\text{coli (ATCC 25 922)}} = 1250 \, \mu\text{g/ml}$$

For *Pseudomonas aeruginosa*, we observed a disorder in the positive control tube, the 0.031% dilution and the 0.062% dilution indicating the presence of a bacterial outbreak. However, no disturbances were observed in the negative control tube, the 0.125% dilution, the 0.25% dilution and the 0.5% dilution.

The MIC value of the chlorobutanol hemihydrate synthesized on the strain of *Pseudomonas aeruginosa* (ATCC 27 853) tested is therefore 0.125%:

$$\text{MIC}_{\text{Chlorobutanol-pseudomonas (ATCC 27 853)}} = 1250 \, \mu\text{g/ml}$$

For *Staphylococcus aureus* (ATCC 25,923) we observed a disorder in the positive control tube, the 0.031% dilution and the 0.062% dilution indicating the presence of a bacterial outbreak. However, no disturbances were observed in the negative control tube, the 0.125% dilution, the 0.25% dilution and the 0.5% dilution.

The MIC value of chlorobutanol hemihydrate synthesized on the strain of *Staphylococcus aureus* (ATCC 25923) tested is 0.125%:

$$\text{MIC}_{\text{chlorobutanol-staphylococcus (ATCC 25 923)}} = 1250 \, \mu\text{g/ml}$$

For Candida albicans, we observed a disorder in: the positive control tube, the 0.031% dilution, the 0.062% dilution and the 0.125% dilution, which indicates the presence of a bacterial outbreak. However, no disturbances were observed in the negative control tube, the 0.25% dilution and the 0.5% dilution.

The value of the MIC of the chlorobutanol hemihydrate synthesized on the candida albicans strain tested is therefore 0.25%:

$$\text{MIC}_{\text{Chlorobutanol-candida}} = 2500 \, \mu\text{g/ml}$$

For *Aspergillus niger*, we observed a disorder in: the positive control tube, the 0.031% dilution, the 0.062% dilution and the 0.125% dilution, which indicates the presence of a bacterial outbreak. However, no disturbances were observed in the negative control tube, dilution at 0.25% and dilution at 0.5%.

The value of the MIC of the chlorobutanol hemihydrate synthesized with respect to the *Aspergillus niger* strain tested is therefore 0.5%:

$$\text{MIC}_{\text{chlorobutanol-aspergillus}} = 2500 \, \mu\text{g/ml}$$

Table VI summarizes the values of the MIC on the strains tested.

**IV. CONCLUSION**

The synthesized chlorobutanol drug substance is of good physicochemical quality. It meets the requirements of the European Pharmacopoeia. The microbiological study revealed an antimicrobial effect on all strains tested (*Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger*) with MIC values close to the data in the literature.
Table IV

The results of the MIC

<table>
<thead>
<tr>
<th></th>
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<th>1250</th>
<th>2500</th>
<th>5000</th>
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<tbody>
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<td>-</td>
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</tr>
<tr>
<td>(CMI)</td>
<td></td>
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<tr>
<td>Pseudomonas aeruginosa</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(CMI)</td>
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<td>Staphylococcus aureus</td>
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<td>(CMI)</td>
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<td>Candida albicans</td>
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References