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# Synthesis, Structural Characterization, and Antimicrobial Evaluation of Dimethyl Methylene Phosphate: An Innovative Phosphorylated Compound with Broad-Spectrum Potential

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**Abstract:** Dimethyl Methylene Phosphate was synthesized and its chemical and antimicrobial properties were investigated. Its structure was corroborated by advanced spectroscopic methods (IR, NMR and UV-Visible spectroscopy), underscoring the important role played by the phosphate group. The compound showed potent antibacterial activity with inhibition zones against Gram-negative bacteria such as *Alcaligenes faecalis* reaching 47 mm. Furthermore, UV-Visible quantitative analysis showed the maximum absorption at 260 nm, affirming its electronic properties. The results establish its potential as a broad-spectrum antimicrobial agent, warranting further exploration into its biological applications and mechanisms of action.

**Key Words:** Dimethyl Methylene Phosphate (DMP), IR spectroscopy, NMR spectroscopy, antimicrobial activity, phosphate compounds

## 1. Introduction

Phosphate-containing compounds play a pivotal role in both biological and chemical systems, serving as integral components in cellular energy transduction, signal transduction pathways, and as scaffolds for advanced drug development. The escalating crisis of antimicrobial resistance has underscored the urgency of discovering innovative compounds capable of targeting resistant pathogens effectively.

Dimethyl Methylene Phosphate (DMP), a novel phosphorylated compound, represents a significant advance in this domain. Its

distinctive structural features, particularly the phosphoryl (P=O) bond, enable unique interactions with bacterial membranes, potentially disrupting essential cellular functions. In this study, we report the synthesis of DMP, provide a comprehensive evaluation of its antimicrobial activity, and investigate its structural and chemical properties using advanced spectroscopic techniques. These findings aim to shed light on the potential of DMP as a new tool in the fight against antimicrobial resistance.

## 2. Experimental

### Chemical Part

#### The synthesis protocol for Dimethyl Methylene Phosphate

Dimethyl Methylene Phosphate (DMP) was synthesized using pulegone as a precursor. In a typical procedure, pulegone (0.35 g, 2.25 mmol) was dissolved in diethyl ether, and diethylamine was added dropwise while stirring to ensure homogeneity. The resulting mixture was phosphorylated by the gradual addition of phosphoric acid (12 mL) under controlled conditions at 60°C for 3 hours. Upon completion, the reaction mixture was cooled to ambient temperature, promoting crystallization of the product. Purification was achieved via recrystallization employing a diethyl ether/benzene solvent system, yielding DMP in high purity as confirmed by analytical techniques.

#### Characterization methods

- **IR Spectroscopy**: The IR spectrum of DMP exhibited key absorption bands corresponding to its functional groups: 2985 $cm^{-1}$ (C-H stretching), 1250 $cm^{-1}$ (P=O stretching), and 1023 $cm^{-1}$ (P-O-C stretching). These findings confirm the successful incorporation of phosphorylated functional groups.

- **NMR Spectroscopy**:
  - *$^{1}H$ NMR*: Notable signals included a triplet at 0.9 ppm (methyl protons), a singlet at 2.1 ppm (methyl group adjacent to C=O), and multiplets between 4.0-5.0 ppm, attributed to methylene groups interacting with polar moieties.
  - *$^{13}C$ NMR*: The spectrum revealed peaks characteristic of distinct carbon environments: 0-50 ppm (aliphatic carbons), 120-150 ppm (aromatic carbons), and 160-180 ppm (carbonyl carbons).
  - *$^{31}P$ NMR*: A single, sharp peak at 10 ppm confirmed the presence of a single phosphorus environment, indicative of the compound's organo-phosphate nature.

- **UV-Visible Spectroscopy**: DMP exhibited maximum absorption at 260 nm, with an extinction coefficient (ε) of 0.127 $L \cdot mol^{-1} \cdot cm^{-1}$. This absorption is consistent with the electronic transitions associated with the phosphorylated moiety, providing

further evidence of the compound's structural framework.

## Biological Part

### Antimicrobial evaluation

The antimicrobial efficacy of Dimethyl Methylene Phosphate (DMP) was evaluated using the disk diffusion method across six clinically-relevant bacterial strains: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella pneumoniae*, and *Alcaligenes faecalis*. Sterile disks impregnated with DMP at concentrations of 1, 2, 5, and 10 mg/mL were applied to Mueller-Hinton agar plates inoculated with bacterial suspensions. Following 24 hours of incubation at 37°C, inhibition zones were measured, providing quantitative data on the compound's antimicrobial potential.

## 3. Results and Discussion

- ✓ **Chemical Characterization**

The chemical structure of DMP was confirmed through a comprehensive suite of analytical techniques:

- **IR Spectroscopy**: The spectrum (Figure 1) revealed prominent absorption bands at 1250 $cm^{-1}$ (P=O stretching) and 1023 $cm^{-1}$ (P-O-C stretching), consistent with the functional groups characteristic of organophosphates.
- **NMR Spectroscopy**: The $^{1}H$ NMR spectrum displayed key signals, including a triplet at 0.9 ppm (methyl protons), a singlet at 2.1 ppm (methyl adjacent to C=O), and multiplets at 4.0–5.0 ppm (methylene groups). The $^{13}C$ NMR spectrum exhibited peaks at 0–50 ppm (aliphatic carbons), 120–150 ppm (aromatic carbons), and 160–180 ppm (carbonyl carbons). A sharp $^{31}P$ NMR signal at 10 ppm confirmed the presence of a single phosphorus environment.
- **UV-Visible Spectroscopy**: DMP exhibited a maximum absorption peak at 260 nm, attributed to electronic transitions within the phosphate moiety, which underscores its optical and electronic properties.

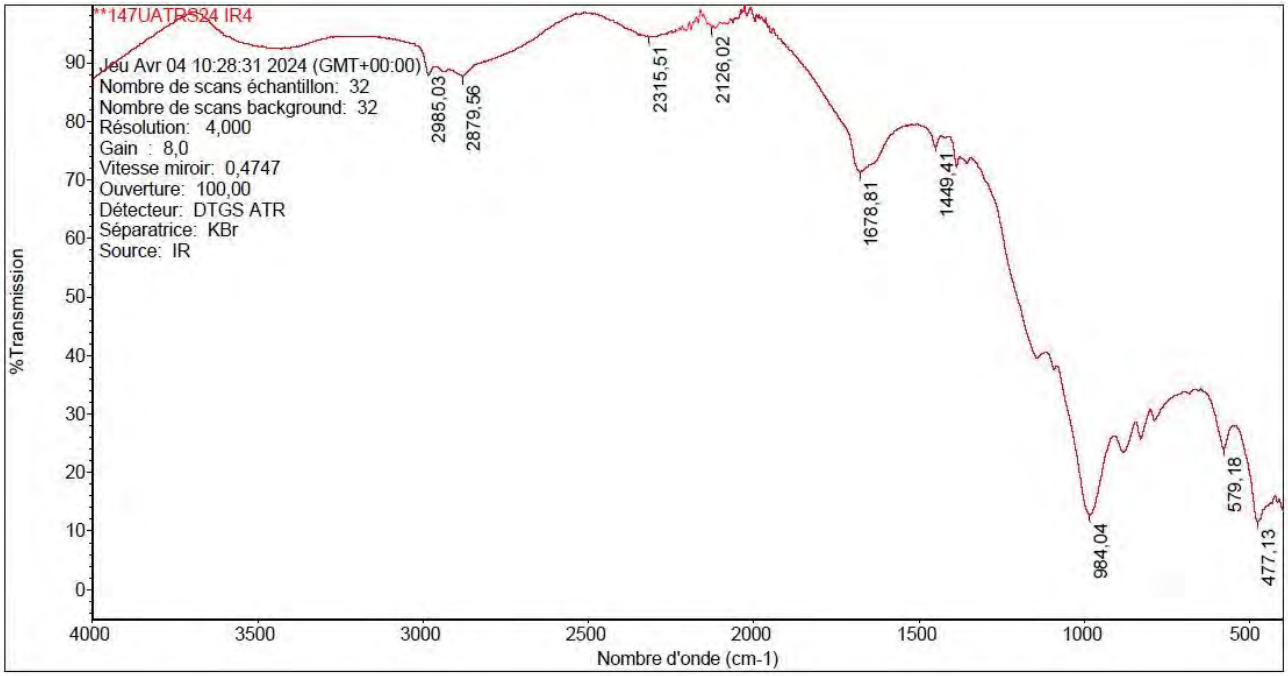


**Figure 1. IR Spectrum of Dimethyl Methylene Phosphate**

- ✓ **Concentration and Solubility:** Quantitative analysis determined DMP's solubility at 0.25 mol/L in ethanol, indicating excellent solubility in polar solvents. This property enhances its applicability in biological systems, where solubility significantly impacts bioavailability and efficacy.
- ✓ **Antimicrobial Activity:** The antimicrobial activity of DMP was assessed across various bacterial strains, with inhibition zones summarized in Table 1. At a concentration of 10 mg/mL, DMP exhibited remarkable efficacy against Gram-negative bacteria, including *Alcaligenes faecalis* (47 mm) and *Escherichia coli* (46 mm). Gram-positive bacteria, such as *Staphylococcus aureus* and *Bacillus subtilis*, exhibited moderate inhibition zones of 38 mm and 32 mm, respectively. The enhanced activity against Gram-negative strains likely results from DMP's ability to penetrate the outer membrane, destabilizing the bacterial cell envelope and interfering with essential intracellular targets. Figure 2 illustrates the significant inhibition zone observed for *A. faecalis*, further demonstrating the compound's broad-spectrum activity. The interaction of the phosphate group with bacterial membranes likely disrupts structural integrity, leading to cellular lysis and death.

**Figure 2. The Inhibition Zone for Six Bacterial Strains (0.25 mol/L Dimethyl Methylene Phosphate on Mueller-Hinton Agar)**

**Table 1. Antimicrobial Activity of Dimethyl Methylene Phosphate**

| Bacterial Strain | Inhibition Zone (mm) |
|---|---|
| *Escherichia coli* | 46 ± 2 |
| *Staphylococcus aureus* | 38 ± 1.5 |
| *Pseudomonas aeruginosa* | 30 ± 1 |
| *Bacillus subtilis* | 32 ± 1.5 |
| *Klebsiella pneumoniae* | 34 ± 1 |
| *Alcaligenes faecalis* | 47 ± 2 |

- ✓ **Mechanism of Action:** The proposed mechanism involves membrane disruption and enzymatic interference. Phosphorylated compounds, such as DMP, integrate into bacterial lipid bilayers, increasing membrane permeability and impairing critical cellular processes. This mechanism provides a plausible explanation for DMP's significant activity against resistant pathogens, including *Pseudomonas aeruginosa*.
- ✓ **Comparative Analysis:** Compared to existing antimicrobial agents, DMP demonstrates several advantages, including high chemical stability, excellent solubility, and consistent efficacy across a range of concentrations. Its robust performance against nosocomial pathogens positions it as a promising candidate for combating antimicrobial resistance. These properties make DMP a valuable addition to the repertoire of anti-

microbial agents with potential clinical applications.

## 4. Conclusion

Dimethyl Methylene Phosphate (DMP) represents a breakthrough in the development of antimicrobial agents. Its significant activity against both Gram-positive and Gram-negative bacteria, combined with its favorable chemical properties, underscores its potential as a versatile therapeutic candidate. This study establishes a robust foundation for further exploration, including optimization of its structure and elucidation of its molecular interactions in biological systems.

Future research should prioritize *in vivo* evaluations, comprehensive safety assessments, and detailed mechanistic studies to support its progression toward clinical applications. The incorporation of DMP into pharmaceutical formulations could provide innovative solutions to combat multi-drug-resistant infections, addressing one of the most pressing challenges in modern healthcare.

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