Egypt. J. Chem. **Vol. 64,** No. 12 pp. 7235 - 7240 (2021)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Synthesis, Characterization of Some Copper (II) Complexes with Ampicillin Derivatives and Study their Biological Activity

Nahed H. Al-Haidery[1], Naeem T. Failli[2] and Hanan A. Al-Hazam[3]

Department of chemistry, Basrah universty, Basrah 6901, Iraq

Abstract

New metal ion complexes of ampicillin were synthesis by two steps, first by N-acylation with benzoyl chloride and sebacoyl chloride, In the second step, the prepared compounds were reacted with cupric ion to form Cu-complexes. FT-IR, ^{1}H NMR, ^{13}C NMR, UV- visible, CHNO-S and a theoretical study was used to distinguish the prepared complexes. The biological efficacy of synthesis compound was found more active against the bacteria.

Keywords: *Ampicillin, Copper (II) complexes, antibacterial activity, AM1 semi-empirical.*

1. Introduction

Ampicillin is a bactericidal antibiotic with wide-spectrum, semi-synthetic, beta-lactam penicillin action. Penicillin-binding proteins (PBP) found on the internal membrane of the bacterial cell wall are inactivated by ampicillin. Inactivation of PBPs intervenes with the cross-linkage of peptidoglycan chains needed for the strength and stiffness of the bacterial cell wall. This interrupts the synthesis of bacterial cell walls and results in the degradation of the cell wall of bacteria and causes cell lists. Ampicillin is stable with a number of beta-lactamases against hydrolysis and can therefore be used in a wide domain of gram-positive and -negative infections.

Ampicillin is known as (2S,5R,6R)-6-[[(2R)-2-amino-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid with molecular formula $C_{16}H_{19}N_3O_4S$[1]. It has biological activity against bacteria[2-6] and microbial[7-8]. Ampicillin has primary amine, which can be used to prepare many chemical derivatives to improve its pharmacological properties such as Schiff base[9] or amide bond [10] and carboxylic group[11]. Amine is also complex with other biological activities such as flavones [12] and formation complexion with Ni (11), Cu (11), Zn (11), and Cd (11) [13].

Copper is also-called biometal since it inters to the formation of some metallic enzymes essential for the normal course of biochemical processes [14]. A person's diet should consistently contain small amounts of copper. Copper complexion with ampicillin in aqueous solution has been studied potentiometers and spectrophotometry. Solution with drugs especially as copper is known to be an active complex-forming agent among drugs[14].

2. Materials

Chemicals and solvents were used directly without any further purification processes. Ampicillin trihydrate was obtained from SDI, Samara, Iraq. Infrared spectra were recorded with a KBR disk in a spectrophotometer (FTIR) in Shimadzu Japan. Elemental microanalysis was performed by Vario EL cube Western Cape; south Africa.1H-NMR spectra were recorded by using deuterated solvent DMSO-d6 by 400MHz type advance ultra shield instrument-Japan in Scool of Chemistry, Tabriz Iran. Spectrophotometric measurements were recorded using a UV-Vis-Spectrophotometer (Spectro scan Shimadzu 80D) in the College of Science – University of Basrah.

*Corresponding author e-mail: m_muntather@yahoo.com.
Receive Date: 25 May 2021, Revise Date: 25 June 2021, Accept Date: 03 July 2021
DOI: 10.21608/EJCHEM.2021.77649.3789

3. Experimental

3.1. Synthesis of compounds

3.1.1. Synthesis of Ampicillin- Benzoyl chloride (1)

6-(2-benzamide-2-phenyl acetamido) methyl -3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo3,2,0heptane-2-carboxylic acid.

A mixture of benzoyl chloride (0.825gm, 3mmol) and ampicillin (1.05gm,3mmol) in 50ml of pure and dry acetone was refluxed for 1hr. with stirring the solid that formed was filtered. Recrystallization of compound was done by using ethanol. the filtrate was used for further reaction (color: yellow, yield 66%) Molecular weight: 467.151 gm/mol, Formula: $C_{24}H_{25}N_3SO_5$.

^{1}H NMR ppm 7.27-7.31 (10H, m, H aromatic), 5.82 (1H,S,H2), 8.8(2H,S,2NH), 3.54 ((2H,S,CH_2N), 1.5(1H,S,2CH_3), 4.54(1H,S,H2), 4.14(1H,s,H5) , 3.7(1H,s,H6), 12.39(1H,S,CO_2H), 13CNMRppm: 136.6 C-1, 129.5 C-2C-6 , 129.2C-3C-5, 129 C-4, 61.1 C2 169.2C1=O,167.8CO, 190.2 (CH2-NH), 47C6, 56.7 C5, 169C7=O, 64.4C3, 79.8C2, 189.7CO_2H, 29.8CH_3, 134.2C1=, 127.5C2=C6=, 128.8C3=C5=, 132.1C4=

3.1.2. Synthesis of Ampicillin- Sebacoyl chloride (2)

6,6‘- (3,6,15,18-tetraoxo-4,17-diphenyl-2,5,16,19- tetraazaicosane -1,20-diyl) bis (3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid)

A mixture of sebacoyl chloride (0.96gm, 4mmol) and ampicillin (2.8gm, 8mmol) in 50ml of pure and dry acetone was refluxed for 1hr. with stirring the solid that formed was filtered. The filtrate was used for further reaction. (Color: yellow, yield 68%), Molecular weight: 892.3499/mol, Formula: $C_{44}H_{56}S_2N_6O_{10}S_2$,

^{1}H NMR: 7.27-7.31(5H,im,ring) 5.82(1H,S,H_2) 5.5 (2H,S,2H) 3.54 ((2H,S,CH_2N), 3.7(1H,S,H_6) 4.14 (1H,S,H_5), 1.3 (6H,S,CH_{2seb}) ^{13}C NMR; 170.9 C=O, 62,9C_2 134C_1 ,129.6$C_{2.6}$,129.2$C_{3,5}$ 127.6C_4, 42,4C-NH,47.5C_6, 169.3 C_7, 90.C_2, 41C_3, 29.7(2CH_3), (38.7$_1$, 3.1$_2$, 27.4$_3$, 27.7$_4$, 27.4$_5$, 27.4$_6$, 31$_7$,38.7) $CH_{2seb.}$

3.1.3. Synthesis of Ampicillin-Sebacoyl copper complexes (3)

5,5,36,36-tetramethyl-14,27-dipheny 2,39,40,41-tetraoxa-6,35-dithia-8,12,15, 26, 29,33-hexaaza-1-cupraheptacyclo[36.1.1.1^{1,3}.0^{4,8}.0^{7,10}.0^{31,34}.0^{33,37}] hentetracontane -9,13,16,25,28,32-hexanone.

Ligand 2 (1mmol) was dissolved in 50ml of pure methanol containing (1.24mmol) of NaOH, solution of cupric nitrate (1mmol) in methanol was added drop wise to the stirring reaction at 25C. Stir the reaction for two hours, filter the product, wash the precipitate with methanol and dry overnight at room temperature to obtain a complex(3). (Colour: green, yield 72%), Molecular weight: 956.53gm/mol, Formula: $C_{44}H_{55}S_2CuN_6O_{10}$, 1HNMR 7.31-7.27(5H,m.ringA)5.82(1H,S,H2)8.81(2H,S,2NH)7.86-7,44(5H,m.ringB)3.54(2H,S, CHN) 3.7(1H,M,H6) 1.33(6H,S,2CH3) 3.68(1H,S,H2) 13CNMR; 169.4C1O, 61.1C2, 136.8C-3, 129.2 C2,6,4 , 127.6C3,-5, 40.2CH2N, 47C6, 57.6C4, 169.3C7=O, 29.7(2CH3), 60C2, 80COO.

3.1.4. Synthesis of Ampicillin- Benzoyl Copper complexes (4)

N-({2-[6-(3,3-dimethyl-7-oxo-6-{[2-phenyl-2-phenylformamido)acetamido] methyl}-4-thia-1-azabicyclo[3.2.0]heptan-2-yl)-1,3,5,7-tetraoxa-4-cupraspiro[3.3]heptan-2-yl]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-6-yl}methyl)-2-phenyl-2-(phenylformamido)acetamide.

Ligand 1 (2mmol) was dissolved in 50ml of pure methanol containing (1.24mmol) of NaOH, solution of cupric nitrate (1mmol) in methanol was added drop wise to the stirring reaction at 25C. The reactions was mixing continuously for two hours, filter the product, wash the precipitate with methanol and dry overnight at room temperature to obtain a complex (**4**). (Colour: green, yield 74%) Molecular weight: 1012.26gm/mol, Formula: $C_{49}H_{52}S_2CuN_6O_{10}$,

^{1}HNMR: 7.27-7.31(5H,im,ring) 5.82(1H,S,H_2) 5.5(2H,S,2H) 3.54((2H,S,CH_2N) 3.7(1H,S,H_6) 4.14(1H,S,H_5), 1.3 (6H,S,CH_{2seb}) ^{13}CNMR170.9 C_1O, 62,9C_2 134C_1 ,129.6$C_{2.6}$,129.2$C_{3,5}$ 127.6C_4, 42,4C-NH 47.5C_6, 169.3 C_7, 90.C_2، 41C_3, 29.7(2CH_3), (38.7$_1$, 3.1$_2$, 27.4$_3$, 27.7$_4$, 27.4$_5$, 27.4$_6$, 31$_7$,38.7) $CH_{2seb.}$

3.2. Biological activity

Antimicrobial activity screening was chosen for all complexes using the spread technique anti at least one of the two bacterial progeny used in the procedure. The solvent utilize was dimethyl sulfoxide (DMSO) for the diffusion well variant method and suitable solvents for the diffusion of chemical products were

used for residual methodologies.

3.2.1. Test-bacteria [15]

The antibacterial effectiveness of synthesis complexes was evaluated against 2 types of bacteria: *Staphylococcus aurous* and Escherichia Coli. The bacteria were cultured after incubation for 24 hours, bacteria suspension (inoculum) was diluted with sterile physiological solution, for the diffusion test, to 10^8 CFU/ml (turbidity McFarland barium sulfate standard 0.5).

The antibacterial activity of synthesis complexes was evaluated against two bacteria species: Staphylococcus aurous and *E. coli*. The bacteria were cultured after incubation for twenty-four hours, bacteria suspension (inoculum) was diluted with sterile physiological solution, for the diffusion test, to 108 CFU/ml (turbidity McFarland barium sulphate standard 0.5).

3.2.2. Agar diffusion well variant

Using sterile cotton swap, the bacterial inoculums were evenly distributed on a sterile MH agar petri dish. In each well, 50 ul of 1 mg / ml from final product was added (7 mm diameter holes cut in agar gel, 20 mm apart). The plates, under aerobic conditions, were incubated for twenty-four hours at 36oC ±1oC. Stacks of bacterial outgrowth was noticed after incubation. The discouragement of bacterial growth was measured in mm.

4. Results and Discussion

The compounds were synthesis are shown in Fig.1. The values obtained from elemental analysis (C, H, N and S content) are in fine accord with those computed form the molecular formula of the synthesis compounds (Table 1).

Cu 's prepared complexes are gray, amorphous solids stable in air and moisture, which decompose without melting. All of them are insoluble in water and other common organic solvents, such as MeOH, EtOH, benzene, hexane, acetone, chloroform, acetonitrile, and ether but soluble in DMSO.

Analyses of IR spectra of the complex have been carried out comparing with the spectrum of IR of ampicillin and synthesized compound have been well studied. The bands present in the ampicillin due to NH2 (3511 cm-1) OH (3447 cm^{-1}) C-H aromatic (3039 cm^{-1}) amide C = O (1774 cm-1). The NH_2 and OH were disappeared in compounds (1, 2) and (3, 4) respectively as shown in Table 2.

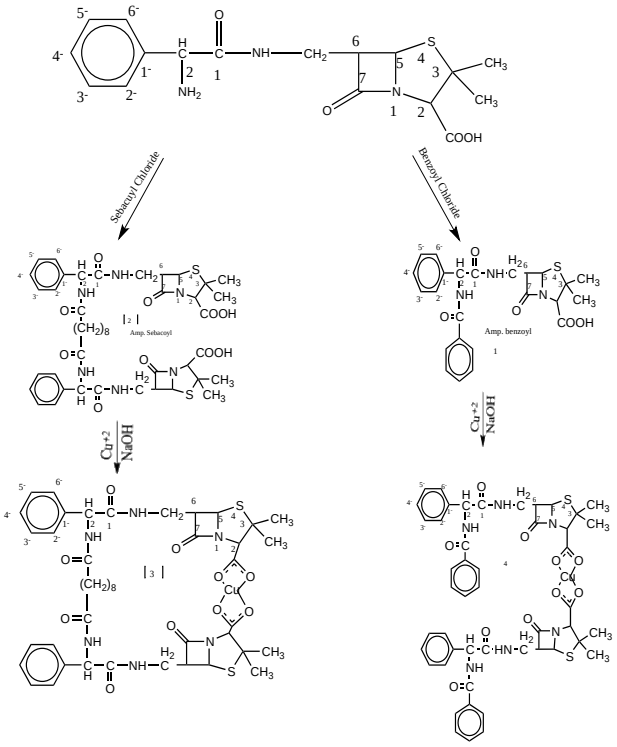

Fig. 1. Synthesis steps for Compounds.

Table 1. The values obtained from elemental analysis (C, H, N and S content)

Comp.	C%	C%	N%	N%	H%	H%	S%	S%
%	found	Cal.	found	Cal.	found	Cal.	found	Cal.
1	61.06	62.1	4.86	5.3	9.29	9.71	7.07	7.4
2	58.9	59.3	4.24	4.71	10.57	10.93	8.06	8.53
3	54.5	53.8	4.6	5.3	9	8.35	4	3.56
4	54.7	54.89	5	6.01	9.12	8.35	6.9	6.59

Table 2. FT-IR spectra of ampicillin complexes

Comp.	NH_2	OH	C-H aromatic	C-H str. Alph	C=O	C=C
Ampicillin	3511	3447	3039	2852	1774	1688
1	-	3445	3049	2970	1774	1696
2	-	3477	3039	2852	1773	1696
3	-	-	3030	2932	1960	1645
4	-	-	3026	2932	1697	1599

U.V -Visible Spectra was measureed. The absorption maxima of the prepared complexes are predominantly transferred some nanometers to the longer wavelength aspect of the absorption band (bathochromic shift) of either of the components in the charge transfer complexion. As shown in Table 3, this alteration was observed between the synthesized compounds and their complexion (13).

Table 3. max. for the compounds

Compounds	1 nm	2 nm
ampicillin	221.5	-
1	211.7	295.4
2	208	298.5
3	211.7	2989.5
4	205.5	304.7

^{13}C NMR the synthesis compounds (1 to 4) were distinguish relying on the addition method by CS-Chem Draw Program. The obtainable value form chemical shifts of ^{13}CNMR [16-19] were also utilized in the characterization.

^{1}H NMR spectra in DMSO-d6 solvent which its appeared at the region 2.2-2.6 ppm. All these spectra showed peaks at the region 8.1-8.2 ppm and 3.7 ppm due to NHCO and CH_3 groups Respectively. CO_2H group at 12.3 ppm in ampicillin and in compounds 2 and 3 which disappeared in compounds 3 and 4 due to complexion as show in fig. 2-5.

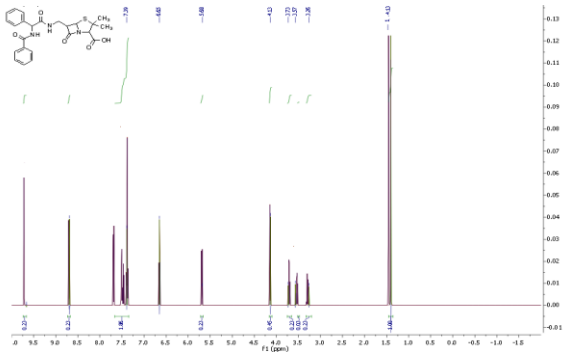

Fig.2. NMR spectra for benzoyl ampicillin

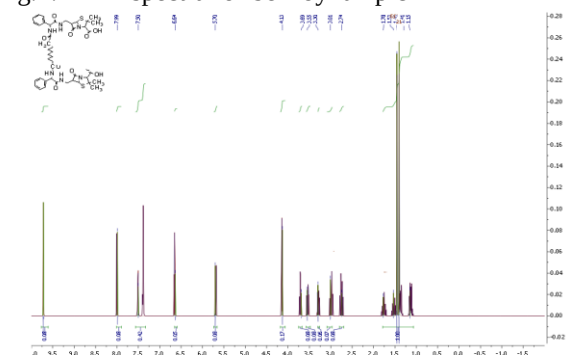

Fig.3 NMR spectra for sebcoyl ampicillin

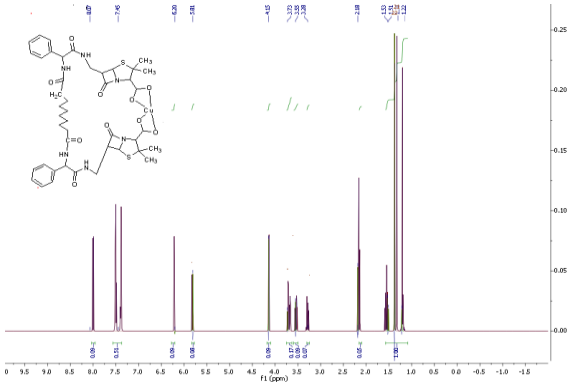

Fig.4.NMR Ampicillin-Sebacoyl copper complexes (3)

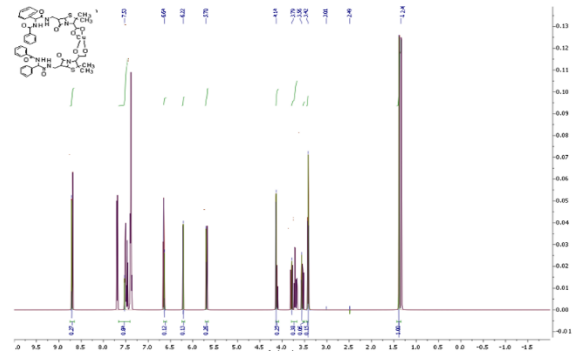

Fig.5.NMR Ampicillin-benzoyl copper complexes (4)

4.1. Biological Results

The compound's antibacterial activities were applied against a certain strain of bacteria. The results showed in Table 4 that synthesis compound 4 was poisonous to bacteria. The compound was found more active against the above microbes.Against the above microbes, the compound was found more active. The relation between the antibacterial activity of those compounds and Streptomycin indicates that the activity of those compounds is nearly identical. Bacterial crops for S. Oh, Aura, and E. Coli was collected from the Basrah University Department of Biology. Oh. Iraq. The bacterial cultures were incubated in nutrient agar for twenty-four hours at 30C^{o} by inoculation.The compound was preserved dry at 25C and dissolved in dimethyl sulfoxide [DMSO] 20 mg / ml. The agar disc-diffusion method evaluated the antibacterial activities of every compound. Mueller Hinton Agar Media [15 cm3] was poured into the petri dishes and left to harden, maintained at 45oC. Poured petri dishes [9 cm] were incubated [105-106 bacteria

per ml] with 50µL of normal isosmotic solution from above-grown media.

Table 4. The antibacterial activities of the compounds [II-VI] Antibacterial data in MIC[µg/ml]

compound	E. Coli [gram –ve]	Staphylococcus aurous[gram +ve]
4	50	50
Streptomycin	12	9

4.2. Theoretical study

As the stable shape of the studied molecules 3 and 4, the AM1 geometry optimization yields a planar structure. As show below in fig. 6 and 7.

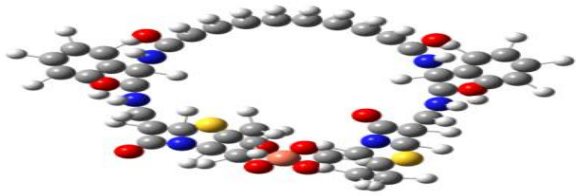

Fig.6. Sebacoyl ampicilline with Cu

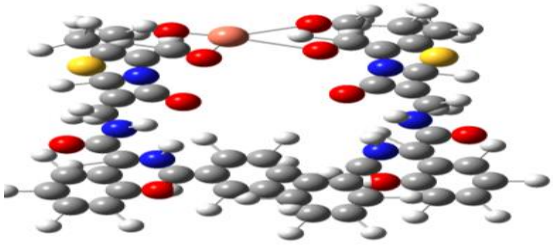

Fig.7. benzoyl ampcilline with Cu

5. Conclusion

This study depict the synthesis of new compound derived from ampicillin by new preparation methods and characterized them by, FT-IR, UV–Vis, ^{13}CNMR, ^{1}HNMR, and element analyses. Copper compound (4) was significantly more effective compared with ampicillin against bacteria S. aurous, and E. coli. This may be due to the presence of the benzene ring in the complexes which enhanced the biological properties by synergistic effect.

6. References

[1] Dee A. L., "Synthesis and biological activity of ampicillin derivative'' B.SC. Thesis, faculty of resource science and technology university Malaysia Sarawak. (2012).

[2] Cherian P. T., Desphanck A., Cheramie M.N., Burhn D. F., Hardle G.J. and, Lee R.E., ''Design,Synthesis and microbiological evaluation of ampicillin tetramic acid hybrid antibiotics'' J. Antibiotic (Tokyo), **70 (1),** 65-72, (2017).

[3] Lampropoulou M., Misiakos K., Paravatou M., Mavridis I.M. and Yannakopoulou K., ''Synthesis of cyclodextrin derivatives with mono saccharide and their binding with ampicillin and selected lectins'' ARKIVOC, **(iii)**, 232-243, (2015).

[4] Cole M., ''Factors affecting,The synthesis of ampicillin and hydroxyl penicillin by the cell-bound penicillin acylase of Escherichia coli Biochem'' J. **115(4)**, 757-764, (1969).

[5] Kucers A. and Bennett N., ''Thesis of antibiotics 3rd ed.William heinemann'' Medical Books, Ltd. London, (1979).

[6] Sekhri L., kadri M.l., Samira C., Senigra M., ''Synthesis of penicillin derivatives and study of their biological antibacterial activities, Biomedical and pharmacology'' J. **(2),** 257-264, (2008).

[7] AL. Ali S., Zowalaty M.I., Hussein M., ''Synthesis, characterization and antimicrobial activity of ampicillin conjugated magnetic nanoantibiotic for medical application'' International j. Nanomedicine, **9(1),** 3801-3814, (2009).

[8] Etran M., Tayhan A., Yulug N. ''synthesis and antimicrobial activities of some new tetrahydro -2H-1,3,5-Hydriazin-2-thion derivatives of ampicillin'' Arch. Pharm., **323**605-609, (1990).

[9] Mohammed M., AL- Mudhafar J. ''Synthesis, characterization and preliminary antimicrobial evaluation of new Schiff-bases of ampicillin and amoxicillin derived from isatin derivatives'' Inter. J. of pharm. and pharmaceutical sci., **8 (5)**, 113-116, (2016).

[10] Shelke j., Meshram G., donger P. ''Synthesis of 2- oxoquinoline-3-carboxamide of ampicillin and amoxicillin as inhibitors of penicillin binding protein 1A of pseudomonas aeruginosa'' **69(4),** 623-628, (2012).

[11] Bartzatt R., Natesa C., ''Synthesis, structural analysis and antibacterial activity of butyl ester derivatives of ampicillin'' chromotherapy, **49**, 213-221, (2003).

[12] Unlusoy M., Altanlar N., Ertan R., ‘‘Synthesis and antimicrobia activities of some new flavonyl pro-drug esters of ampicillin’’ Turk J. of Chem., **29**, (2005).
[13] Bravo A., Anacona R., ‘‘Synthesis and characterization of metal complexes with ampicillin’’ J. Coordination chemistry, **44**, 173-118, (1998).
[14] Lapshin S.V., V. Alekseev G., **‘‘**Copper(II) complexion with Ampicillin, Amoxicillin, and Cephalexin’’ Russian J. of Inorg. Chem., **54**, 1066-1069, (2009).
[15] Smania A., Monache F. D., Smania E. F. A., Cuneo R.S., ‘‘Antibacterial activity of steroidal compounds isolatedfrom Ganoderma applanatum (pers.) pat (Aphyllophoro-mycetideae) fruit body’’ Int. J. med. mushrooms, **1,** 325-330, (1999).
[16] Stothers J. B., ‘‘^{13}C NMR Spectroscopy’’ Academic Press, New York, 1972.
[17] Leyden D. E., Cox R. H., ‘‘Analytic Application of NMR’’ Chapter 5 (John Willey and Sons, New York, 1977.
[18] Abraham R. J., Fisher J., Loftus P., ‘‘Introduction to NMR Spectroscopy John Willey and Sons’’ New York, 24 (1978).
[19] Dyer J. R., ‘‘Application of Absorption Spectroscopy of Organic Compounds Prentice-Hall’’ Inc, Englewood Cliffs, London, 30 (1965).