The New C-2,C-3 Substituted Heterocyclic Derivatives of L-Ascorbic acid: Synthesis, Characterization, and Bacterial Activity

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Abstract

New Schiff bases derivatives $[IV]_{a-e}$ is prepared via condensation of D-erythroascorbic acid with *p*-substituted aldehydes in dry benzene. To obtain these derivatives, the 5,6-*O*-isopropylidene-L-ascorbic acid[I] was chosen as starting material, compound prepared from the reaction of L-ascorbic acid as starting material. Compound[I] was prepared from the reaction of L-ascorbic acid with dry acetone in the presence of hydrogen chloride. The esterification of hydroxyl groups at C-2 and C-3 positions with excess ofethyl α –chloroacetate in the presence of sodium acetate produce acorresebonding ester [II] , which was condensed with hydrazine hydrate to give new hydrazide [III] . The new Schiff bases $[IV]_{a-e}$ were synthesized by reaction of acid hydrizide with different p-substituted benzaldehyde in dry benzene . The new 1,3-oxazepine derivatives $[V]_{a-e}$were obtained by Diels-Alder reaction of Schiff bases with phthalic anhydride in dry benzene (Scheme 1) . All the synthesized compounds have been characterized by melting points , FTIR and ^{1}HMNR (of some of theme) spectroscopy. The biological activity of synthesized compounds was examined against two types of bacteria; G(+) and G(-).

Key words : L- ascorbic acid, Hydrazied, Schiff bases, 1,3-Oxazepine.

الخلاصة

حضرت قواعد شف جديدة من تكاثف D–ارثرو حامض اسكوربيك مع الديهايداتاروماتية معوضة في موقع بارا . تم اختيار 5، 6– *O* –ايزوبروبلدين–L– اسكوربيك اسد [I] كمادة اولية، والذي تم تحضيره من تفاعل L– اسكوربيك اسد كمادة اولية مع الاسيتون الجاف في وجود كلوريد الهيدروجين. تم تحويل مجاميع الهيدروكسيل في C–2 و C–3بتفاعلها مع اثيل كلورواسيتات بوجود خلات الصوديوم ليعطي الاستر الجديد [II] ، والذي تم تكثيفه مع الهايدرازين المائي ليعطي مركب الهايدرازايد[III]. قواعد شف الجديدة $[IV]_{a-e}$ حضرت من تفاعل مركبات الهايدرازايد مع بنزالديهايدات المعوضة في موقع بارا في البنزين الجاف . اما مشتقات 1، 3 – اوكسازبين الجديدة $[V]_{a-e}$ تم الحصول عليها من تفاعل الاضافة لقواعد شف مع انهيدريد الفثالك في البنزين الجاف مع التصعيد العكسي (الشكل 1) . شخصت المركبات المحضرة بقياس درجات انصهارها وبواسطة طيف FTIR وطيف ^{1}HNMR للبعض منها درست الفعالية البايولوجية للمركبات المحضرة ضد نوعين من البكتريا واظهرت النتائج فعالية بايولوجية تراوحت بين العالية والمتوسطة والواطئة ضد نوعين من البكتريا (G–) *Echerichia coli* ,(G+) *Staphylococcus*

الكلمات المفتاحية: حامض الاسكوربيك ، قواعد شف، الهايدرازايد، 1، 3– اوكسازبين .

Introduction

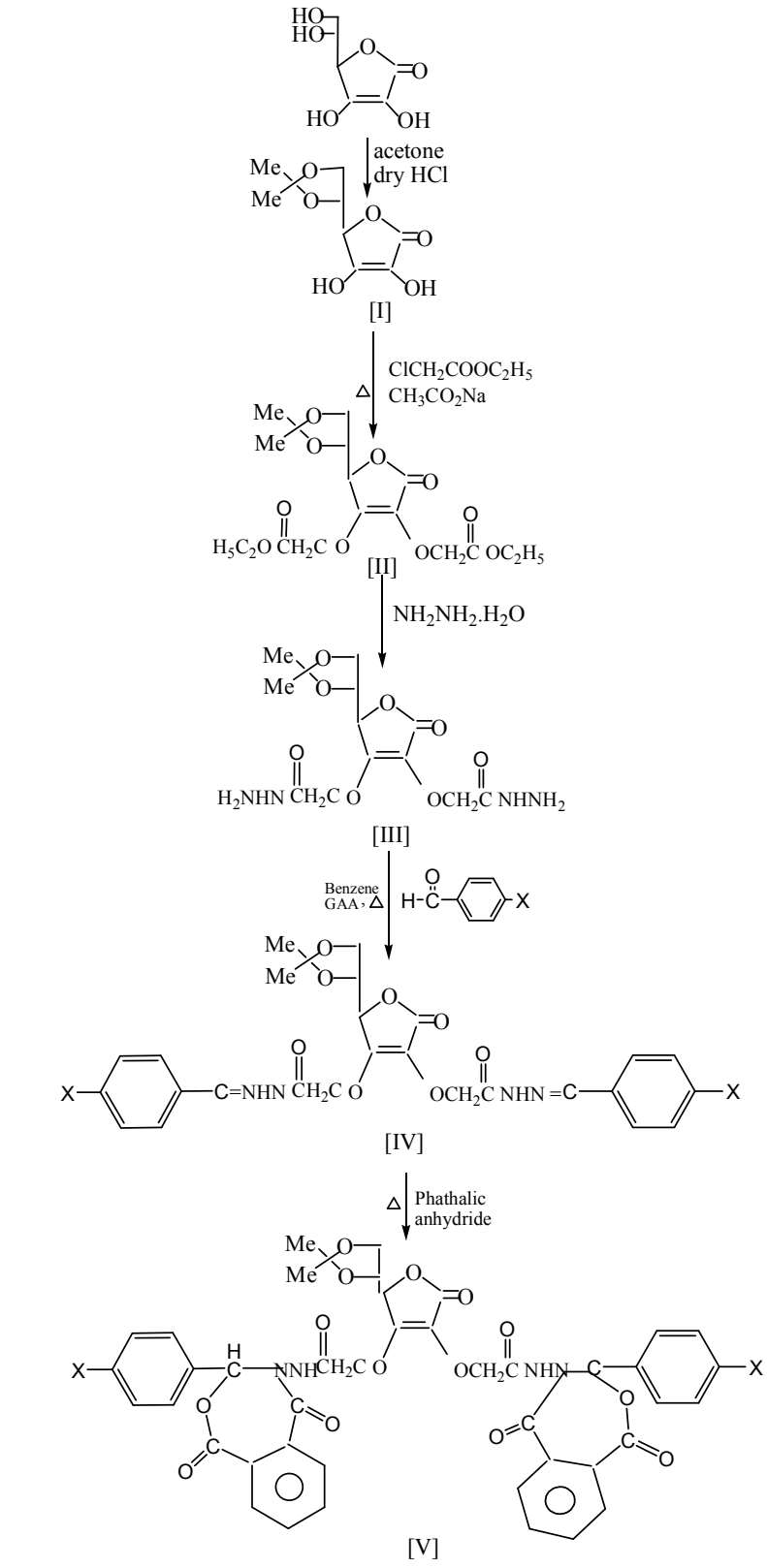
L-ascorbic acid is one of the natural antioxidant present in biological system because of its activity to attack the free radicals and other reactive oxygen species, as the literatures point to the great role which ascorbic acid plays to prevent a number of diseases and its importance in food industry[1,2].

Hydrazides derivatives have growing importance because of the wide spectrum of their biological applications like antibacterial, antitumoral, anti-inflammatory, antifungal and antitubercular agents[3,4].

Hydrazones possessing an azomethine –NHN=CH- proton constitute

an important class of compound for new drug development[5,6], and the imine group played a good intermediate in the organic synthesis of many heterocyclic compounds, as in1, 3-Oxazepine derivativesis[7].

1, 3-Oxazepine non-homologuos seven member rings, that contains oxygen at position 1 and nitrogen at position 3. Oxazepine is used in the medical field and has been much chemically and biologically studied. Many workers synthesized and studied the oxozepine derivatives mentioned as in the literates[8,9].



X= 4-OH , 4-NO_2 , 4-Cl , 4-OCH_3 , 4-Br

Scheme(1)

Experimental

MATERIALS : All the chemicals were supplied from Merck , GCC and Aldrich Chemicals Co. and used as received .

Techniques : FTIR spectra were recorded using potassium bromide discs on a Shimadzo (Ir prestige-21) FTIR spectrophotometer .^{1}HNMR spectra were carried out by company : Bruker , model: ultra shield 300 MHz , origin : Switzerland and are reported in ppm(δ), DMSO was used as a solvent with TMS as an internal standard . Measurements were made at chemistry department, Al-albyat university , Uncorrected melting points were determined by using Hot-Stage, Gallen Kamp melting point apparatus.

SYNTHESIS METHODS

Preparation of 5,6-*O*-isopropylidene-L-ascorbic acid[I]:

This compound was prepared from the reaction of L-ascorbic acid with dry acetone in a acidic media , following Salomonmethode [10].

Synthesis of 2,3-*O*-diethylacetate -5,6 – *O*-isopropylidene-L-ascorbic acid[II]:

A mixture of compound [I] (0.01mole), ethylchloroacetate (0.02mole) and sodium acetate (0.06mole) in (10mL) ethanol was heated under reflux for 4hrs , then cooled and poured into water. The resulting of a white crystals solid was filtered off, washed with water, dried and recrystallized by ethanol to give compound [II]. m.p (60-62)^{0}C, yield 87%.

Preparation of 2,3-*O*-dihydrazide methylene-5,6-*O*-isopropylidene-L-ascorbic acid[III]:

Compound [II] (0.01mol) was dissolved in minimum amount of ethanol. hydrazine hydrate(0.002 mol) was added slowly and the mixture was heated under reflux for 6hrs. Then the mixture was cooled and the glow yellow solid was filtered, and recrystallized from petroleumether. m.p(220-222)^{0}C, yield 78%.

Synthesis of Schiff bases [IV]$_{a-e}$

A mixture of new hydrazide compounds [III] (0.01 mol) , p-substituted benzaldehyde (0.02 mol) , dry benzene (15 mL) was refluxed for 6hrs . The solvent was evaporated under vaccum and the residue crystallized from chloroform. The physical data of all Schiff bases are listed in Table (1).

Synthesis of1,3-oxazepine [V]$_{a-e}$

A mixture of equimolar amounts (0.01mol) of Schiff bases [IV]$_{a-e}$ and phthalicanhydriedein dry benzene as a solvent was refluxed for 8 hrs , the solvent was removed and the resulting colored crystalline solid recrystallized from petroleum ether to obtained 1,3-oxazipenes [IV]$_{a-e.}$The physical properties of all synthesized 1,3-oxazepines are listed in Table (1) .

Results and Discussion

The first step employs the protection of the hydroxyl groups at C-5 and C-6 positions in L- ascorbic acid with acetal leading to formation compound 5,6-*O*-isopropylidene-L-ascorbic acid [I] using dry acetone in acidic media, following Salomon[10] method. The FTIR spectrum showed a broad stretching band in the region (3240-3074) cm^{-1} for O-H vinylic, stretching bands between (2993-2890) cm^{-1} for C-H aliphatic, a stretching band at 1751 cm^{-1} due to C=O of Lactone ring, a stretching band at 1663 cm^{-1} for C=C and stretching bands at (1141) cm^{-1} for C-O stretching .

This is followed by reaction of the hydroxyl groups at C-2 and C-3 positions with two mole of ethyl chloro acetate in basic media. The FTIR spectrum for compound [II] has confirmed the formation of compound [II] by disappearance the bands for (O-H) of compound [I] and exhibited appearance of a stretching band at (1707) cm^{-1} for (C=O) of the ester group. Stretching bands are between(2924-2834)cm^{-1} for C-H aliphatic group.

Condensation of one mole of compound [II] with two mole of hydrazine hydrate give a new hydrazide

[III] ,which is characterized by its melting point, FTIR spectrum, which showed the following absorption bands: C=O of amide group at (1677) cm^{-1}, bands in the regions (3450, 3200, 3194) cm^{-1} due to asymmetric and symmetric stretching vibration of NH , NH_2 groups. Also the spectrum showed band at (2987-2853) cm^{-1} assignable to thestretching of aliphatic (C-H).The ^{1}HNMR spectrum (in DMSO) showed a sharp singlet signal at δ 1.38 ppm for protons of CH_2 groups , a doublet signal at δ 2.28ppmfor NH-NH_2 protons,singlet signal at δ 3.10ppm for three protons of CH_3acetal groups and asingle at 3.79 ppm for NH group[11].

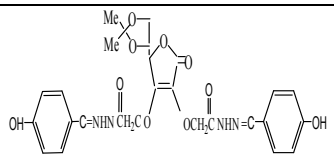
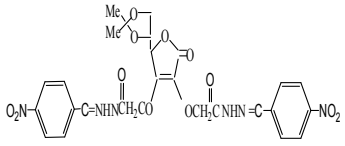
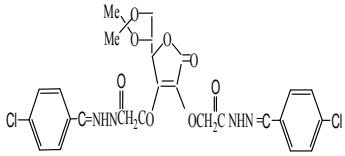
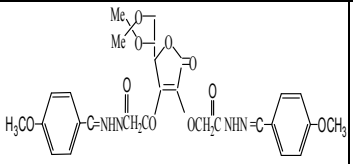
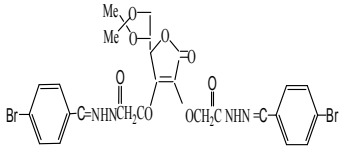
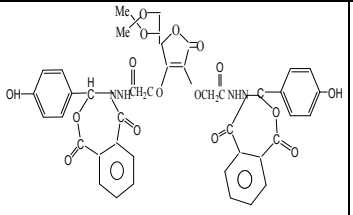
New Schiff bases $[IV]_{a-e}$ are prepared via condensation of hydrazide compound [III] and p- substituted benzaldehyde in dry benzene. These Schiff bases $[IV]_{a-e}$ are characterized by their melting, FTIR and ^{1}HNMR Spectroscopy . FTIR absorption-spectra showedthe disappearance of absorption bands due to NH_2 and C=O groups of the starting materials together with appearance of new absorption stretching band for imine C=N group.

The other data of functional groups which are characteristics of these compounds are given in Table 2.

The ^{1}HNMR spectrum (in DMSO), Figure (1) of compounds $[IV]_c$ showed a sharp signal at δ 2.60 ppm for protons of methylene groups and a signal at δ 3.15ppm which could be for three protons of CH_3acetal groups, doublet of doublets at δ(6.57-7.41) ppm could be attribiuted to eight aromatic protons. Furthermore, a sharp signal for N-H proton was absorbed at δ 8.28 ppm .

The 1,3-oxazepine derivatives [V]a-e were obtained by Diels-Alder reaction of Schiff bases[IV]a-e with phthalic anhydridesin dry benzene.The characteristic FTIR absorption bands of these compounds, was confirmed from the disappearance of band due to C=N of schiff base and other peaks characterized of cyclic anhydride of the starting materials together, with appearance of new bands for oxazepine ring. All the spectral data of FTIR spectroscopy of these compounds are listed in Table (2).The ^{1}HNMR spectrum of compound $[V]_e$ (in DMSO), Figure (2) , showed a singlet signal of N-CH proton absorbed at δ (8.51) ppm, the aromatic ring protons appear at the range (δ 6.90-8.16) ppm, a singlet signals at δ(3.61) ppm for CH_3acetal groups, finally, a singlet signal at δ (1.98) ppm that could be attributed to the proton of CH_2 groups.

Table 1: Physical properties of synthesized compounds [IV]a-e and [V]a-e

Comp. No.	Nomencalture	Structural Formula	Molecular Formula	M.P^{0}C	Yield%	Color
$[IV]_a$	2,3-*O*-bis[(4`-hydroxy phenyl) semicarbazone methylene]- 5,6-*O*-isopropylidene-L-ascorbic acid		$C_{26}H_{28}N_4O_{10}$	198-200	78	Yellow
$[IV]_b$	2,3-*O*-bis[(4`-nitro phenyl semicarba -zone methylene]- 5,6-*O*-isopropylidene-L-ascorbic acid		$C_{26}H_{26}N_6O_{12}$	118-120	75	Green-yellow
$[IV]_c$	2,3-*O*-bis[(4`-chlorophenyl) semicarbazone methylene]- 5,6-*O*-isopropylidene-L-ascorbic acid		$C_{26}H_{26}N_4O_8Cl_2$	106-108	72	Dark Yellw
$[IV]_d$	2,3-*O*-bis[(4`-methoxyphenyl) semicarbazone methylene]- 5,6-*O*-isopropylidene-L-ascorbic acid		$C_{28}H_{32}N_4O_{10}$	164-166	55	Orange
$[IV]_e$	2,3-*O*-bis[(4`-bromophenyl) semicarbazone methylene]- 5,6-*O*-isopropylidene-L-ascorbic acid		$C_{26}H_{26}N_4O_8Br_2$	132-134	50	Red-brown
$[V]_a$	2,3-*O*-bis[(4`-hydroxyphenyl) 2,3-dihydro benz [1,2e][1,3]oxazepine-4,7-diones-methyl ene]- 5,6-*O*-isopropylidene-L-ascorbic acid		$C_{42}H_{40}N_4O_{16}$	182-184	80	Pale Orange

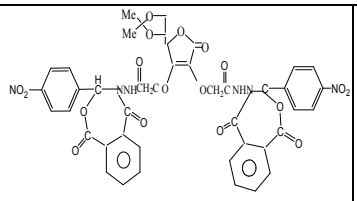
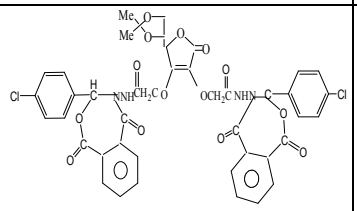
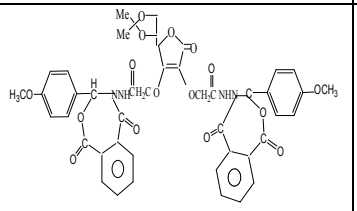
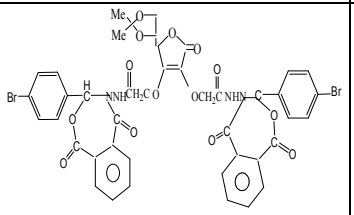
$[V]_b$	2,3-*O*-bis[(4`-nitro phenyl) 2,3-dihydro benz [1,2e] [1,3] oxazepine-4,7-diones-methylene]- 5,6-*O*-isopropylidene-L-ascorbic acid		$C_{42}H_{38}N_6O_{18}$	124-126	82	White
$[V]_c$	2,3-*O*-bis[(4`-chlorophenyl) 2,3-dihydro benz [1,2e][1,3]oxazepine-4,7-diones-methyl ene]- 5,6-*O*-isopropylidene-L-ascorbic acid		$C_{42}H_{38}N_4O_{14}Cl_2$	176-178	75	Brown
$[V]_d$	2,3-*O*-bis[(4`-methoxy phenyl) 2,3-dihydro benz [1,2e][1,3]oxazepine-4,7-diones-methyl ene]- 5,6-*O*-isopropylidene-L-ascorbic acid		$C_{44}H_{44}N_4O_{16}$	168-170	70	Pale Brown
$[V]_e$	2,3-*O*-bis[(4`-bromophenyl) 2,3-dihydro benz [1,2e][1,3]oxazepine-4,7-diones-methyl ene]- 5,6-*O*-isopropylidene-L-ascorbic acid		$C_{42}H_{38}N_4O_{14}Br_2$	140-142	50	Brown

Table 2: Characteristic FTIR absorption bands of synthesized compounds

Comp. No.	νC-H aliph.	νC-H arom.	νC=N	νC=C arom.	CO-NH	Other
$[IV]_a$	2800	2960	1639	1558	1681	4-OH:3450
$[IV]_b$	2839	2920	1616	1556	1678	4-NO_2:1385-1360
$[IV]_c$	2872	2940	1618	1558	1666	4-Cl:800
$[IV]_d$	2852	2985	1602	1577	1689	4-OCH_3:1259
$[IV]_e$	2850	2922	1620	1589	1681	4-Br:750
$[V]_a$	2889	2990		1585	1687	4-OH:3336
$[V]_b$	2859	2924		1585	1681	4-NO_2:1384-1359
$[V]_c$	2850	2960		1593	1683	4-Cl:760
$[V]_d$	2820	3007		1585	1697	4-OCH_3:1282
$[V]_e$	2812	2993		1585	1683	4-Br:750

Table 3 : Antibacterial activity of the synthesized compounds $[IV]_{a-e}$-$[V]_{a-e}$

Comp. No.	*E.Coli*(G-)	*Staphlococcu saurus(G+)*	Comp. No.	*E.Coli*(G-)	*Staphlococcus aurus(G+)*
$[IV]_a$	-	+ +	$[V]_a$	++	+++
$[IV]_b$	++	+	$[V]_b$	+	+++
$[IV]_c$	-	-	$[V]_c$	++	+++
$[IV]_d$	+ +	++	$[V]_d$	++	++
$[IV]_e$	+	+ +	$[V]_e$	+++	+++

Key to symbols: Highly active = + + +(mor than)15 mm.

Moderately active = + +(11-15) mm. and Slightly active = + (5-10) .

Biological Activity

The antibacterial activity of the synthesized compounds was performed according to the agar diffusion method[12]. The synthesized compounds were tested against *E.coli* and *Staph. aureus*. Each compound was dissolved in DMSO to give concentration 1ppm. The plates were then incubated at 37°C and examined after 24 hrs. The zone of inhibition formed were measured in millimeter and clarity as in Table 3. All the compounds exhibit the highest or low biological activity against both the organisms . Most of the 1,3-oxazepin compounds showed a high inhibition against gram(+) becteria that could be related to the heterocyclic ring. While the Schiff bases compounds showed low-moderiate activity against two types of the bacteria gram(+) and garm (-). Finally, the Schiff base $[IV]_e$ did not show any biological activity towards bacteria gram(+) and garm(-) .

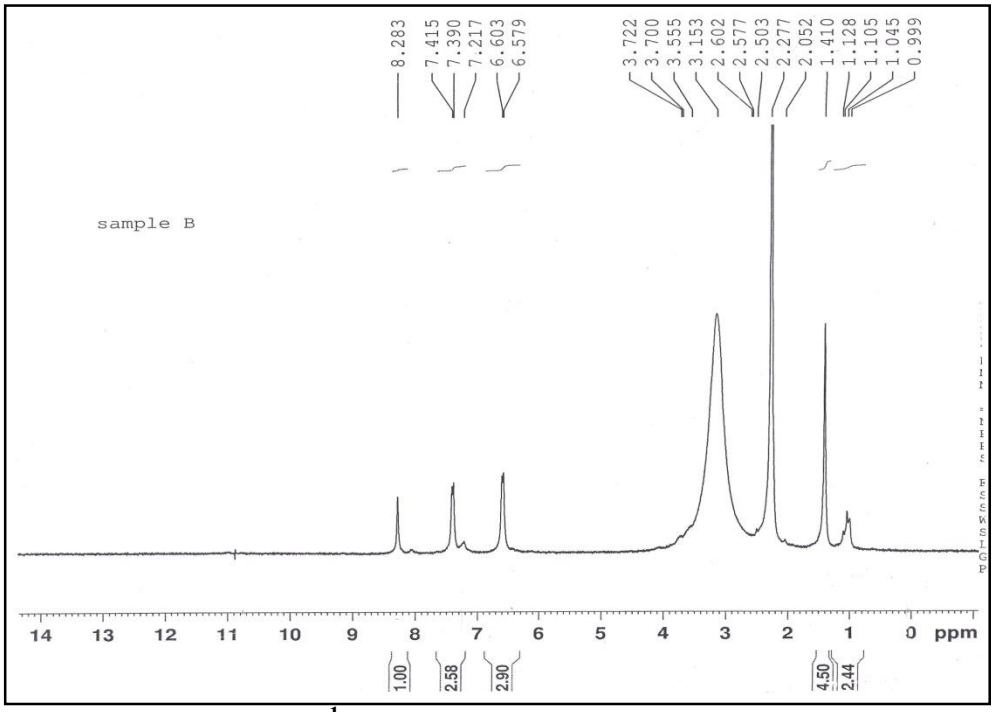


Figure (1)^{1}HNMR spectrum of compound $[IV]_c$

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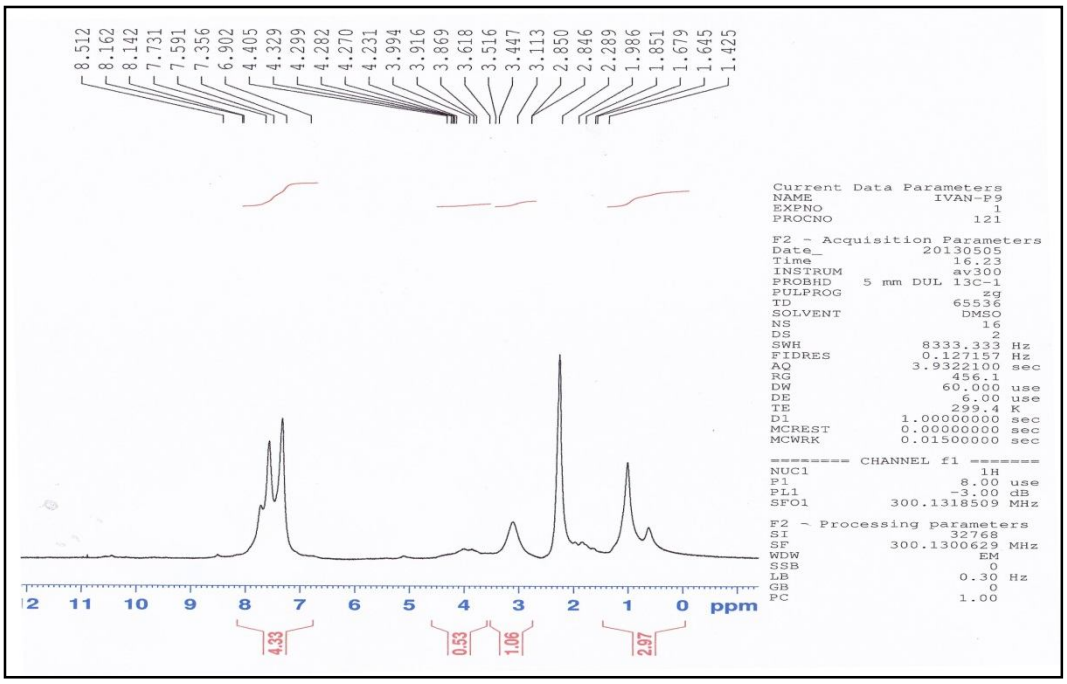

Figure (2)^{1}HNMR spectrum of compound $[V]_e$

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