International Journal of Chemistry and Applications.
ISSN 0974-3111 Volume 4, Number 3 (2012), pp. 149-157

http://www.irphouse.com

Synthesis, Characterization and Antioxidant Study of Some New Compounds of Gallic Acid Derivatives

[*]Hanan A. Alhazam, Zainab Shakir Abdullah[**], and Nahed Al-haidery

Department Of Chemistry, College Of Science
University of Basrah, Basrah, Iraq.
[*]E-mail:hananalhazam1965@yahoo.com,
and zainab1976@yahoo.com

Abstract

A series of gallic acid (3,4,5-trihydroxy benzoic acid) derivatives are prepared by reactions of (3,4,5-trihydroxy benzoyl chloride) with NH_2ArXY; where X, Y=(4′-Cl, 2′-Me**(1)**); (3′-Cl, 2′-Me**(2)**); (3′-Cl, 4′-Me**(3)**); (3′-Br**(4)**); (4′-Br**(5)**); (2′-Me, 5′-Me**(6)**) and (2′-Me, 4′-Me**(7)**). The new compounds were characterized spectroscopy, theoretically and an antioxidant study. Were $AM_1$and PM_3semi-empirical calculation methods were confirmed that the compounds existed as trans form.

Keywords: Gallic Acid, Antioxidant Activity, Spectroscopy, Theoretical Study.

Introduction

An antioxidant is a compound that stops an oxidation reactions from occurring such as vitamin E which prevents radical reaction that can cause cell damage by terminate radical chain mechanism [1,2]. A free radical is a molecule with one or more unpaired electrons in the outer orbital. Many of these free radicals are in the form of reactive oxygen and nitrogen species, these can occur, due to oxidative stress brought about by the imbalance of the bodily antioxidant defense system and free-radical formation [3]. Oxidative stress has been linked to cancer, aging, ischemic injury, inflammation and neurodegenerative diseases (Parkinson's and Alzheimer's). Reactive oxygen species(ROS) such as superoxide radical ($CO2^-$), hydroxyl radical(OHC), peroxyl radical (ROOC) and nitric oxide radical(NOC), attack biological molecules such as lipids, proteins, enzymes, with aging, atherosclerosis carcinogenesis [4] and may lead to the development of chronic diseases related to the cardio and

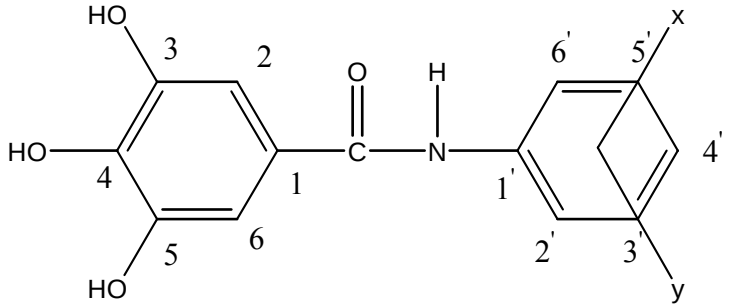
cerebrovascular systems [5]. Antioxidants at relatively low concentrations prevent or retard the oxidation of biological macromolecules [6]. Gallic acid (3,4,5-trihydroxy benzoic acid) ,is one of the most common phenolic acids. Gallic acid is known to have anti-inflammatory, anti-mutagenic, anticancer and antioxidant activity [7-13]. It also seems to have antifungal, antiviral [14] and antibacterial properties [15]. Gallic acid was found to show cytotoxicity against cancer cells without harming healthy cells, and it is used as a remote astringent in cases of internal haemorrhage. It has been found very beneficial in uterine, pulmonary, and nephritic haemorrhages. It is used to treat albuminuria and diabetes [14].All these properties make gallic acid a pharmacologically important used compounds.In our present study, were perpered seven compunds derivatives of gallic acid and evaluted as antioxidant activities.

Experperiment Work

Preparation

New seven compounds were parepared in this study based on literture [16,17].Gallic acid of amount of 0.01mole or its sodium salt is mixed with 0.01mole of thionyl chloride.The mixture is heated under a small refluxe condenser for 30 minutes then colded. A solution of 0.01mole of amine in 30ml ofbenzene is added, and the mixture is warmed on the steam bath for 20 minutes. The benzene solation is deconted into spearation funnel and washed successively with 2ml of water, 5 ml of 5% hydrochloried acid, 5ml of 5% sodium solation and 2ml of water. The benzene is evapoated and the amid is recrystallized from water or ethanol to obtain the seven compound as described in Table 1.

Table 1. Characteristic of compounds.



Comp. No.	X	Y	Molecular weight	Yield	Formula form	Color	Melting point
1	4-Cl	2-Me	293	60	$ClC_{14}H_{12}NO_4$	Brown	182°C
2	3-Cl	2-Me	293	60	$ClC_{14}H_{12}NO_4$	Brown	142°C
3	3-Cl	4-Me	293	60	$ClC_{14}H_{12}NO_4$	Brown	118°C
4	3-Br	H	324	65	$BrC_{13}H_{10}NO_4$	Yellow	218°C
5	4-Br	H	324	65	$BrC_{13}H_{10}NO_4$	Yellow	148°C
6	2-Me	5-Me	273	70	$C_{15}H_{15}NO_4$	Brown	195°C
7	2-Me	4-Me	273	75	$C_{15}H_{15}NO_4$	Brown	185°C

Physical Measurements

The IR-spectra were recorded on 9 SHMADZ 8400 FT-IR spectrophotometer. The melting points were measured by a Gallenkampointapparatus, and were uncorrected. The NMR spectra were also obtained in deutrated solation DMSO using Bruker 300MHz.Type advance Mltrasheild instrument in central laboratories of the Institute of earth and environment scienceof the university of Al al-Bayt, Jordan.

Theoretical Calculation

All geometrical optimized carried out by AM1 and PM3 method. AM1 is an improvement over MNDO, even though it uses the same basic approximation. It is generally the most accurate semi-empirical method and is the method of choice for most problems. PM3 is a re-parameterization of AM1, which is based on the neglect of diatomic differential overlap (NDDO) approximation. NDDO retains all one-center differential overlap terms when Coulomb and exchange integrals are computed. PM3 differs from AM1 only in the values of the parameters. The parameters for PM3 were derived by comparing a much larger number and wider variety of experimental versus computed molecular properties. Typically, non-bonded interactions are less repulsive in PM3 than in AM1. PM3 is primarily used for organic molecules, but is also parameterized for many main group elements.The AM1 and PM3 semi–empirical methods of theHyperChem. 6.01 program were utilized to compute heat of formation (ΔH) [18-20]. All theoretical computations were performed in a Pentium IV PC.

Determination of antioxidant activity

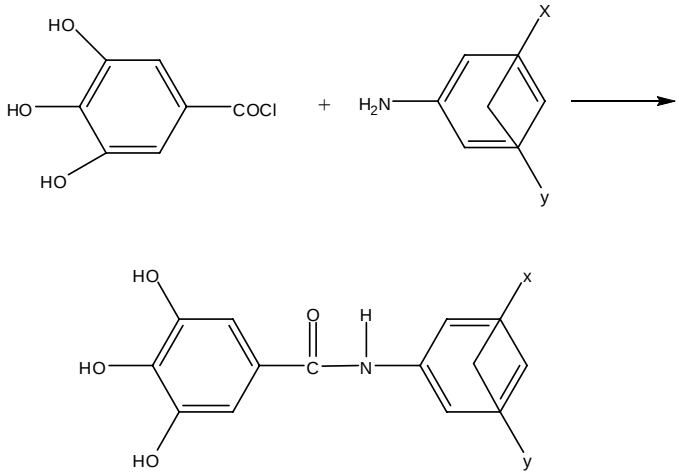
Antioxidant activity of Gallic acid and its derivatives was determined according to the β-carotene bleaching [21-23] with the following modification. A solution of β-carotene wasprepared by dissolving 2mg of β-carotene in 10ml of chloroform,1ml of this solution was then pipet into a round-bottom rotary flask containing 20mg of linoleic acid and 0.2g of Tween 20. After removing the chloroform under vacuum using a rotary evaporator at 30^{o}C, 50 ml of aerated distilled water was added to the flask with manual shaking. Aliquots 5ml of this prepared emulsion were transferred into tubes containing 0.2ml of samples (Gallic acid,α-tocopherol, BHT, prepared derivatives and control consisted of 0.2 ml of methanol instead of the extract. As soon as the emulsion was added to each tube, the zero time absorbance was read at (470nm). The samples were then subjected to thermal autoxidation at 50^{o}C in a water bath. Subsequent absorbance readings were recorded at 15min intervals until the color of the β-carotene in the control sample had disappeared at 105min. The extent of inhibition of the absorbance is related to the concentration of antioxidant compounds. All samples were taken in triplicate. The degradation rate of extracts was calculated according to zero order reaction kinetics. Antioxidant activity (AA) was calculated as percentage of inhibition relative to the control using the following equation:

$$AA = \left[1 - \left(\frac{A_j - A_t}{A_j^* - A_t^*}\right)\right] \times 100 \quad (1)$$

Where A_j: measured absorbance value of sample at zero time.A_t: measured absorbance value of sample after incubation (105min) at 50°C.A_j^*: measured absorbance value of control at zero time. A_t^*: measured absorbance value of control after incubation (105min) at 50°C.

Results and Discussion

In this work, Gallic acid derivatives have been prepared from the reaction of 3,4,5-tri hydroxyl benzoyl chloride with substituted aromatic amine H_2NArYX as illustrated in Scheme 1.



Scheme 1.

FT-IR Spectral Analysis

The FT-IR spectrums of compounds were obtained and effective peaks were compounded with that of the standard Gallicacid. The FT-IR spectrum of the standard Gallic acid [24] contains thirteen major peaks at the range of 3307, 2960, 2931, 2831, 2865, 1639,1509,1420,1453,1097,777,663, 602 &462cm^{-1}whereas the FT-IR spectrum of the new compounds show new peaks at 3480-3419 cm^{-1} as a multiple band related to the N-H bond of secondary amide in solid samples. Multiplied bands were observed since the amide group can bond to product dimmers with S-cis conformation (Fig.1) or polymers with an S-trans conformation (Fig.2) [25].

All the IR spectra of Gallic acid derivatives showed strong-medium bands at 1610-1680cm^{-1} and 1590-1600cm^{-1}which are the characteristicof the C=O and C=C ring stretching respectively. Strong band at 1400-1530 cm^{-1}which is the characteristic of C-N stretching.

O-H stretching band appears in the range 3300-3250 cm^{-1} as a broad band due to intermolecular hydrogen bond.

A broad, medium band also absorbed in the 800-650cm^{-1} region which results from the out of plane N-H wagging.

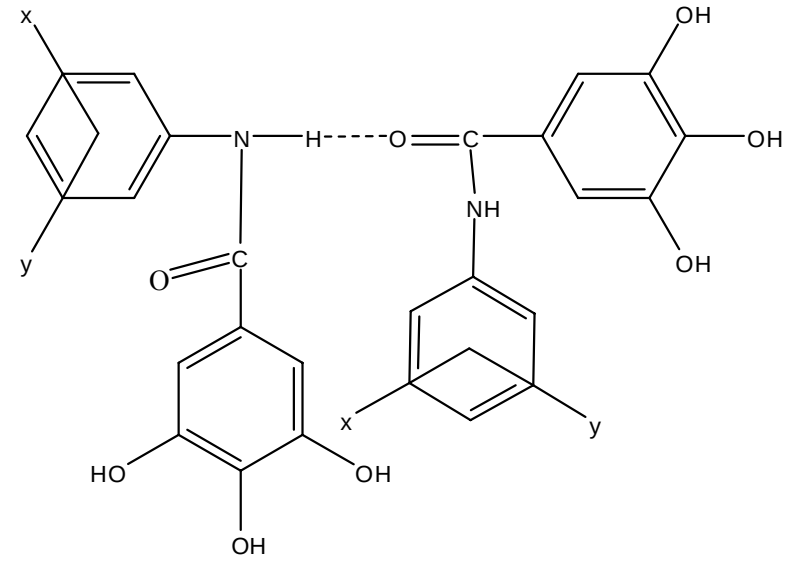


Figure 1. S-trans form

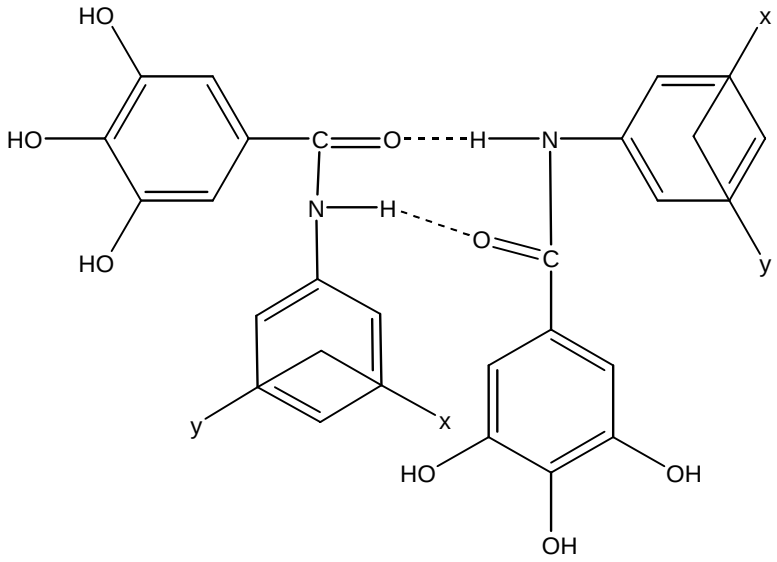


Figure 2 S-cis form

Theoretical Calculation

AM_1 and PM_3 semi-empirical calculation methods have shown that the S-trans form is more stable than the S-cis form and the heat of formation of S-transis smaller than S-cis form as a billeted in Table 2 .

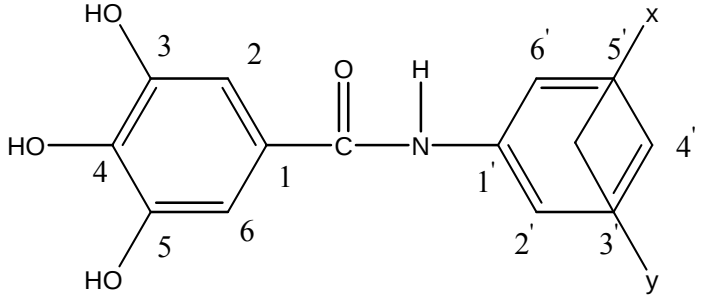
Table 2. Heat of formation (ΔH) (kcal/mole) using AM1 and PM3 method.

Comp. No.	AM1	PM3	AM1	PM3
	S-trans Form		S-cis Form	
1	-101	-100	-120	-125
2	-100	-102	-124	-126
3	-101	-104	-115	-118
4	-99	-103	-102	-109
5	-120	-124	-125	-127
6	-99	-102	-103	-109
7	-98	-102	-103	-108

.^{1}H NMR

^{1}HNMR spectra for thestudiedcompounds showsKetoform in the range of 10.2-9.8ppm for H on N atom.Phenolic protons absorbed down field to the range of about 9.9-9.5ppm.All spectra showed atripled signal in the region 6.93-6.91ppm due to the protons at locations2and 6. The aromatic ring system showed multiplied signal with in the region 6.9-7.9ppm .^{1}H NMR data of these aromatic protons are summarized in Table 3.

Table 3. Chemical shifts (ppm) and coupling constant (H_z) of aromatic protons for



Comp. No.	X &Y	H2′	H3′	H4′	H5′	H6′	Me
1	4′-Cl, 2′-Me	_	7.4	_	7.20 d, J5′6′=7.5	7.60d, J5′6′=7.5	2.1
2	3′-Cl, 2′-Me	_	_	7.30 d, J4′5′=6.5	7.18t, J4′5′=6.5	7.19d, J5′6′=6.5	2.2
3	3′-Cl, 4′-Me	7.80	_	_	7.15d , J5′6′=7	7.44d , J5′6′=7	2.3
4	3′-Br	7.80	_	7.55d, J4′5′=6.5	7.17t J4′5′=6.5	7.55d, J5′6′=6.5	_
5	4′-Br	7.70d, J2′3′=7	7.50d, J2′3′=7	_	7.5d, J5′6′=7	7.7d, J5′6′=7	_
6	2′-Me, 5′-Me	_	7.09d J3′4′=7	6.85d, J 3′4′=7	_	7.33	2.12 2.34
7	2′-Me, 4′-Me	_	7.08	_	7.02d, J5′6′= 6.5	7.12d, J5′6′= 6.5	2.12 2.34

Antioxidant Study

β-carotene bleaching activity which is used to assess the power of the new prepared compounds as antioxidants, is presented in Fig.3 with the reference compounds,Gallic acid,α-Tocopherol and BHT. The linoleic acid free radical attack the highly unsaturated β-carotene. The presence of different antioxidants can hinder the extent of β-carotene bleaching by neutralizing the linoleate-free radical and other free radical formed in the system [23].Accordingly, the absorbance decreased rapidly in samples without antioxidant (control) whereas, in the presence of an antioxidant, samples retained their color, and thus absorbance, for a longer time. The antioxidant activity of the new compounds is shown in Table4.

Table4. Antioxidant activity of prepared compounds (1-7) and α- Tocopherol, BHT and Gallic acid as reference compounds.

Sample	AJ	At	A*J	A*t	AA
αTocopherol	0.629	0.376	0.600	0.178	40
BHT	0.667	0.387	0.600	0.178	33
gallic acid	0.631	0.389	0.600	0.178	42
1	0.630	0.399	0.600	0.178	45
2	0.612	0.345	0.600	0.178	36
3	0.666	0.429	0.600	0.178	43
4	0.650	0.306	0.600	0.178	18
5	0.697	0.398	0.600	0.178	29
6	0.672	0.332	0.600	0.178	19
7	0.692	0.300	0.600	0.178	7

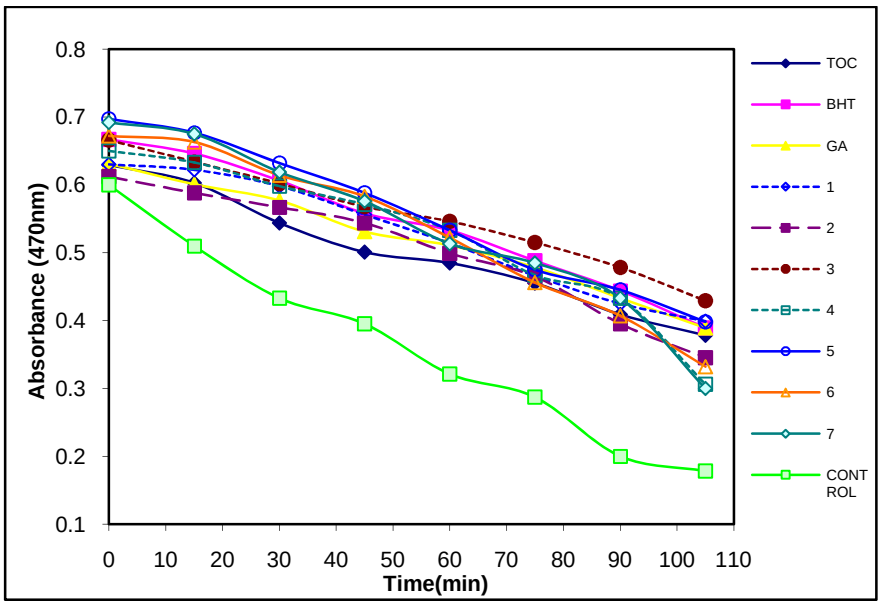


Figure.3. Antioxidant activity of prepared compounds (1-7) and α- tocopherol, BHT and Gallic acid as reference compounds.

Conclusion

In new derivative compound, the highest antioxidant activity was observed in compound number 1, 3, 2, 5, 6, 4 and 7respectively. The compounds (Gallic acid derivatives) that have S-trans forms are more stable than the S-cis form because the heat of formation of S-transis smaller than S-cis form.

References

[1] J. Gorzynski "Organicchemistry" McGraw-Hill companies, Inc., 2006.

[2] F. Carey "Advance organic chemistry" third ed., McGraw-Hill companies, Inc., 1996.

[3] C. Teow, North caroline state unviersty, **9,** 2005.

[4] O. Tevlik, Grasas Y Aceltes, **1**, 86,2010.

[5] K. Mahnaz,H.Mannan,C.Mina,K.Zahra,A.Yousef,H. Abbas and RMohammed, J. of Biotechnology,**6**, 11432009.

[6] R. Apak, K. Güçlü, B. Demirata, M. Özyürek, S. E. Çelik, B. Bektaşoğlu, K. I. Berker, D. Özyurt, J.*Molecules*, **12**, 1496 , 2007.

[7] T.Gichner, F.Pospisil, J.Veleminsky, V.Volkeova,&L. Volke,.Folia Microbiol., **32**, 55-62, 1987.

[8] M. T. Huang, R. L. Chang, A. W. Wood, H. L.Newmark, J. M.Sayer, H. Yagi, et al. Carcinogenesis, **6**, 237242,1995.

[9] M.Inouc, R. Suzuki, Sakaguchi, N., Zong, L., Takeda, T., Ogihara, Y., et al. Biological and Pharm. Bulletin, **18**, 1526-1530, 1995.

[10] B. H.Krores, A. J. J. Van den Berg, H. C. Q.Ufford, H. Van Dijk, & R. P. Labadie,. PlantaMedica.**58**, 499-504, 1992.

[11] H. L.Mandsen, & G.Bertelsen, Trends Food Sci. Technol, **6**, 271-277, 1995.

[12] 12.S. Mirvish, A.Cardesa, L.Wallcave, & P.Shubik,.Journal of the NationalCancer Institute, **55**, 633-636, 1975.

[13] N.Nakatani, M. T. Huang, C. T. Ho, & C. Y. Lee (Eds.). ACS SymposiumSeries(507, pp. 72-86). Washington, DC: American ChemicalSociety, 1992.

[14] Phytochemicals http://www.phytochemicals.info/phytochemicals/gallicacid.php

[15] Medical Dictionary-http://www.springboard4health. com/notebook/dict_g.html.

[16] A.shriner "The Systematic Identification of Organic Compounds" 4th.ed.,JohnWiley and sons, Inc.,1956.

[17] A.Vogel"Practical organic chemistry" 4th. ed.,Longmans, Greenand Co LTD 1957.

[18] M. Dewar, E. Zoebisch, E. Healy and J. Stwart, J. Am. Chem. Soc., **107**, 3902, 1985.

[19] M. Dewar and K. Dieter, J. Am. Chem. Soc. **10**, 8075, 1986.

[20] J. Stwart, J. ComputeChem. **10**, 221,1989.

[21] G. Marco, J. Am. Oil Chem. Soc. **45**, 594 ,1968.

[22] M. Al-Saikhan, L. Howard and J. Miller,. J. Food Sci. **60**, 341 , 1995.
[23] G. Jayaprakasha, R. Singh and K. Sakariah, Food Chem. **73** 285 2001.
[24] S. Meenakshi, D. ManickaGnanambigai, S.Tamilmozhi,M. Arumugam and T.Balasubramanians,.GlobalJ.Pharmacology,**2**, 59,2009.
[25] R. Silverstein "Spectrometric identification of organic compounds " 7^{th}. ed., John Wiley and Sons, Inc.,2005.