Synthesis and Analgesic Properties of Some Azosalicylate Compounds

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Abstract: Azo dyes are widely used colorants in consumer products such as leather, textiles, agriculture, cosmetics and in laboratories as indicators, and these type of compounds used recently in the pharmaceutical preparations. This paper describes the synthesis of some azo dyes derived from salicylic acid. The structure of the products was confirmed by IR, ^{1}H-NMR, ^{13}C-NMR spectroscopy. The synthesized dyes were investigated for their analgesic properties using Swiss albino mice.

1. Introduction

Salicylates are the class of compounds that are widely valued for their pain killing, antipyretic and anti-inflammatory properties **[1]**. The most commonly known and used salicylates are salicylic acid (also called 2-hydroxybenzoic acid), aspirin (acetylsalicylic acid -ASA) and sodium salicyclates. They are used extensively for the relief of headache, inflammation, arthritis pain, and some are employed in the treatment of heart attacks and strokes in the elderly **[2]**.

Azo dyes constitute the largest group of commonly available dyes and pigments. Due to their colour, azo dyes are used as pigments, indicators of solvent polarity of molecular environments and chemical environments **[3]**. They are also widely used as histological stains and in the colori-metric analysis of pharmaceuticals **[4]**.

Inflammation is a complex biological response of vascular tissues to harmful stimuli. The stimulus may be thermal (heat or cold), chemical (foreign substances, foreign organisms, drugs), or mechanical (trauma). The classical signs of an inflammatory process are Rubor (redness), Tumour (swelling), Calor (heat), Dolor (pain) and function laesa (loss of function). Analgesics are the drugs which relieves the pain. Anti-inflammatory agents are the agents which relieves the inflammation. In market various analgesic and anti-inflammatory formulations and dosage forms are available of aspirin, paracetamol, ibuprofen etc.**[5,6]** Therefore, attempts have been made to synthesize azo dyes compounds derived from salicylic acid and evaluate their biological activity as analgesic agents.

2. Experimental part

2.1. General

All the reagents and solvents are reagent-grade quality and were purchased from Merck and Sigma-Aldrich, and used without further purification for solid materials and with twice distillation for liquid materials. The uncorrected melting points were determined on a Gallenkamp Thermal Point Apparatus. Infrared spectra (in KBr pellets) were recorded on a FTIR 8400S SHIMADZU (Japan) in College of Pharmacy, Basrah University. The ^{1}H-NMR and ^{13}C-NMR spectra in DMSO-*d6* were measured at 300 MHz using Bruker AC 200 FT-NMR spectrometer using TMS as an internal reference, (Greece).

2.2. Synthesis of compounds

The azo dyes compounds, p-(m-Hydroxyphenyl azo) salicylic acid; **2a**, p-(p-Nitrophenyl azo) salicylic acid; **2b** and p-(p-Carboxyphenyl azo) salicylic acid; **2c** were prepared with two reactions by the same method.

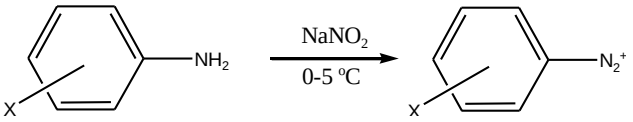
2.2.1. Diazotization [7]

Twenty mmol of amines p-aminobenzoic acid (2.74 g), m-aminophenol (2.18 g) or p-nitroaniline (2.76 g) was dissolved in 10 ml of 2 M HCl. The solutions were cooled to 0-5 ^{o}C in an ice-bath and maintained at this temperature. Sodium nitrite (20 mmol, 1.38 g) in water (10 ml) then added drop wise for each mixture with continues stirring for 10 min at the same temperature to give three diazonium salts, **1a**, **1b** and **1c**.

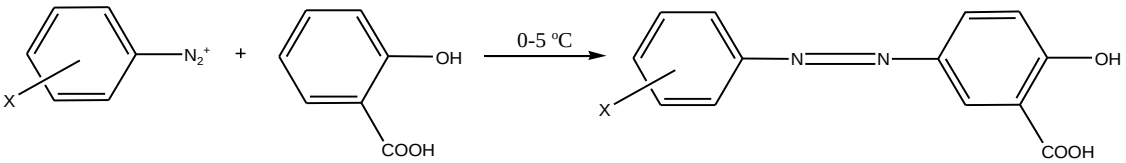
2.2.2. General procedure for preparation of azo dyes [7]

The diazonium solution was added portion wise to the coupling component solution of salicylic acid (20 mmol, 2.76 g) (Scheme 1) in 10 ml of water with sodium carbonate (3 mmol, 0.32 g) dissolved in 15 ml of water. During the procedure the pH value was maintained within 9-10 and the temperature at 0-5^{o}C. The mixture was stirred for 6 hrs, then the pH value was

decreased to about 6. The mixture was kept overnight. The precipitated crude dyes were collected by filtration, washed with water, ethanol and acetone. Table 1 shows the characterizations of the prepared compounds.



X: m-hydroxy; **1a**
p-nitro; **1b**
p-carboxy; **1c**



X: m-hydroxy; **2a**
p-nitro; **2b**
p-carboxy; **2c**

Scheme 1: Preparation of azo dyes compounds

Table 1: Physical properties of the azo dyes compounds

Comp.	Molecular Formula	M. Wt. (g/mole)	m. p. (°C)	Crystals color	Yield (%)	R_f	Eluent
2a	$C_{13}H_{10}N_2O_4$	258.23	177-180	Dark brown	72.8	0.81	Ethyl acetate: ethanol (8:2)
2b	$C_{13}H_9N_3O_5$	287.23	174-176	Dark orange	69.28	0.74	
2c	$C_{14}H_{10}N_2O_5$	286.24	284-287	Light brown	86.11	0.79	

2.3. Analgesic activity

2.3.1. Animals

Swiss albino mice (18-24g) of either sex were bred in the Faculty of Pharmacy Animals House. Food and water were provided from animal's house. Experiments were carried out between 10.00 and 13.30 h. The mice were divided into 5 groups (n=6) in each group for the tested compounds and controls groups. The controls groups were divided into two groups, positive control group was given standard drug (aspirin) and negative control group was given water.

2.3.2. Anti-Nociceptive test (Hot Plate Test)

Male and female albino mice showing reaction time of 20 sec. to thermal stimulus of 55 ± 3 °C were selected. The mice groups were given dose 50 mg/kg. b. o. of compound for treated groups and 0.2 ml water to the negative control group and 50 mg/kg. b. o. aspirin to positive control group. The reaction time for control and treated mice were recorded after 1.30 and 3.00 h of drug administration. **[8]**

3. Results and Discussion

3.1. IR spectra

The IR spectra for all azo compounds were performed by KBr disc method. Table 2 represents the data of the important bands of the IR spectra of the three prepared compounds. All IR spectra of the compounds showed a broad band in the range 3251-3240 cm-1 which characteristic of O-H stretching vibration of hydroxyl of phenolic and carboxylic groups. All three compounds showed a strong band in the range 1711-1662 cm^{-1} attributed to C=O stretching of carboxyl group of acid moiety.**[9]**

All prepared compounds showed a strong-medium bands in the ranges 1446-1442 cm^{-1} and at

1249-1246 cm^{-1} and 1161-1153 cm^{-1}, the first band is attributed to the N=N stretching vibration of azo group and the second bands are attributed to C-N and C-O stretching vibrations of the two fragments, as shown in Figures 1-3 and Table 2.

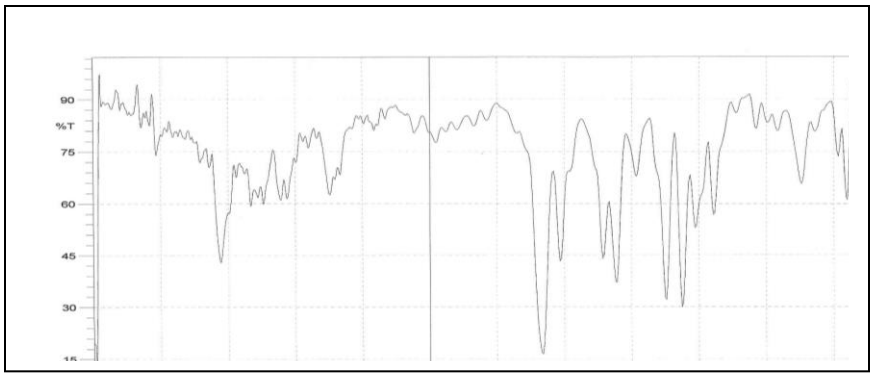


Figure 1: FT-IR spectrum of 2a

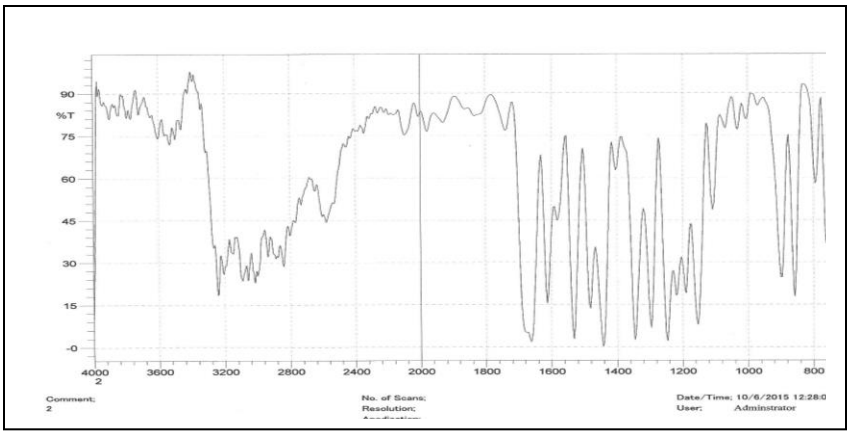


Figure 2: FT-IR spectrum of 2b

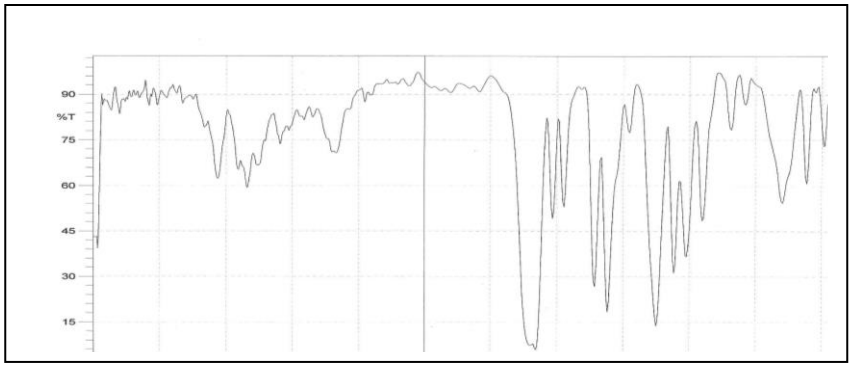


Figure 3: FT-IR spectrum of 2c

Table 2: IR spectra data of azo compounds

2a	*2b*	*2c*	*Assignment*
3244 m	3240 m	3251 m	O-H stretching
3062 w 3020 w	3089 m 3012 m	3074 m 3005 w	C-H stretching aromatic
1662 s	1711 s	1701 s	C=O stretching
1612 m 1585 w	1612 m 1581 w	15612 m 1577 m	C=C stretching of aromatic rings
1442 m	1442 s	1446 s	N=N stretching of azo group
1249 s	1246 s	1246 s	C-N stretching
1157 m	1153 s	1161 m	C-O stretching
698 m	694 s	671 s	C-H bending aromatic

3.2. ^{1}H-NMR spectra

The 1H-NMR chemical shift values for compounds **2a-2c** in DMSO-*d6* solution are listed in Table 3. The ^{1}H-NMR spectra of these compounds show multiplet signals in the regions 6.87-8.40 ppm which assigned to the protons of aromatic rings **[10]**. All title compounds exhibit broad signals at the range 11.36-12.14 ppm which can be attributed to the proton carboxylic of salicylic acid fragment. The hydroxyl protons of salicylic fragment for all compounds appeared, in low-field, as a singlet signal at 9.75-10.11ppm due to hydrogen bonds**[11]**. ^{1}H-NMR spectra of compounds 2a and 2c showed singlet and broad signals at 5.01 ppm and 11.10 ppm, respectively, which can be ascribed to the hydroxy group proton of m-hydroxypheny fragment and carboxy group proton of p-carboxyphenyl fragment, respectively. Figures 4-6 represent the ^{1}H-NMR spectra of the azo compounds.

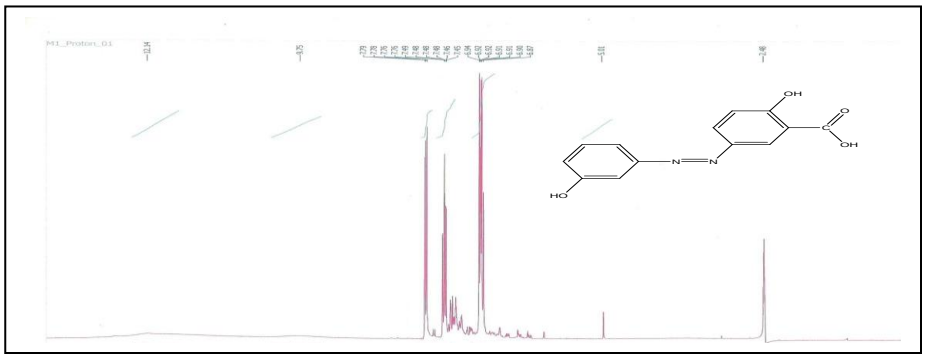


Figure 4: ^{1}H-NMR spectrum of compound 2a

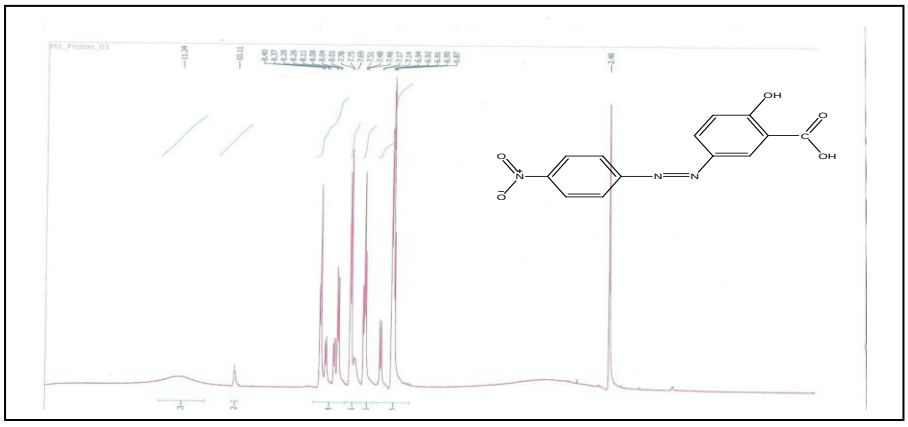


Figure 5: ^{1}H-NMR spectrum of compound 2b

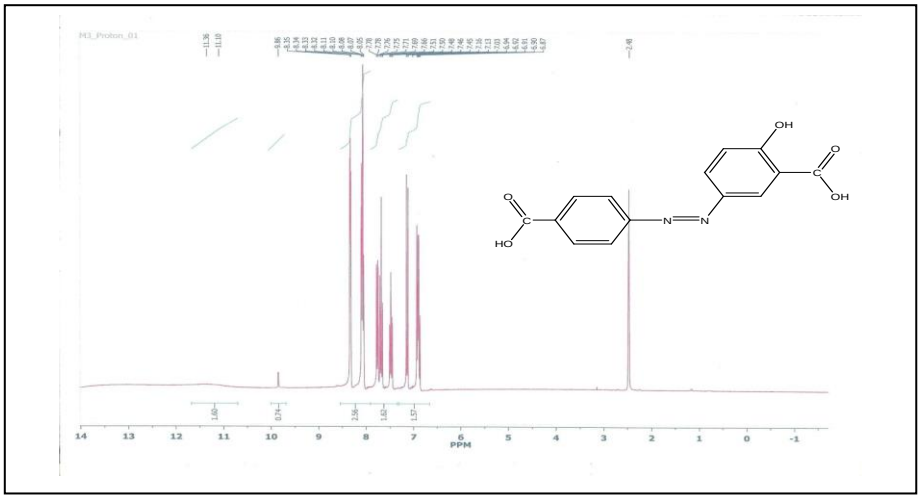


Figure 6: ^{1}H-NMR spectrum of compound 2c

Table 3: ^{1}H-NMR data of the azo dyes compounds

Compd.	δ ppm				
	-COOH salicylic	-OH salicylic	-OH	-COOH	Aromatic
2a	12.14 (br) 1H	9.75 (br) 1H	5.01 (s) 1H	-	6.87-7.79 (m) 7H
2b	11.24 (br) 1H	10.11 (s) 1H	-	-	6.87-8.40 (m) 7H
2c	11.36 (br) 1H	9.86 (s) 1H	-	11.10 (br) 1H	6.87-8.35 (m) 7H

br: Broad, s: Singlet, m: Multiplet

3.3. ^{13}C-NMR spectra

^{13}C-NMR spectra were measured in DMSO-d6 solution and the chemical shifts are presented in Table 4. All of the possible carbon signals of two azo compounds (2b and 2c) are observed in the ^{13}C-NMR spectra, as expected.

In the ^{13}C-NMR spectrum of 2b, the signal appeared at 172.33 ppm was assigned to carboxyl carbon atom (COOH, C-7). A signal at 161.55 ppm was assigned for phenolic carbon (C-2). A signal at 155.63 ppm was referred to C-8 which attached to nitro group. Two signals at 148.56 and 144.87 ppm due to C-11 and C-5, respectively which attached to nitrogen of azo group.**[12]** Other aromatic carbons showed following signals 136.08, 130.69, 129.58, 127.37, 123.69, 119.61, 117.52, 113.33 ppm, as shown in Figure 7.

The ^{13}C-NMR spectrum of 2c gave the following signals, downfield signals at 172.33 and 171.72 ppm assigned to carboxyl carbons C-7 and C-14, respectively. Phenolic carbon (C-2) gave signal at 167.15 ppm. Two carbon atoms attached to the nitrogens of azo group appeared as two signals at 152.25 and 144.80 ppm. Other aromatic carbons showed the following signals, 136.07, 132.55, 130.69, 130.29, 127.56, 122.58, 119.60, 118.87, 117.51 ppm, as shown in Table 4 and Figure 8.

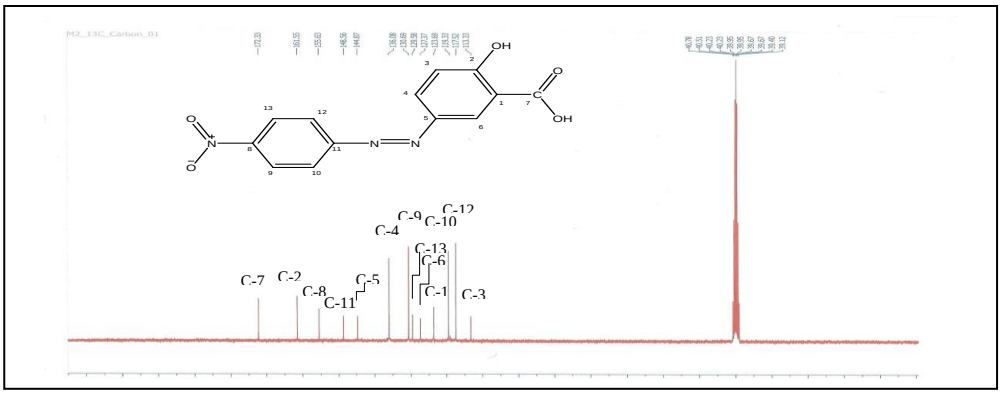


Figure 7: ^{13}C-NMR spectrum of compound 2b

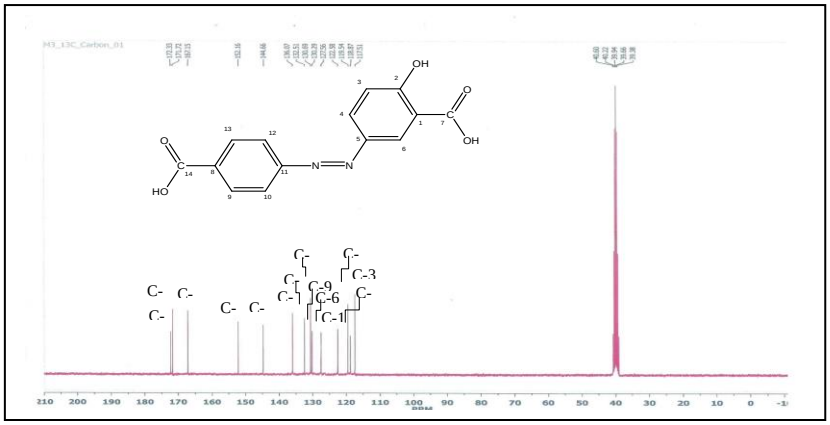


Figure 8: ^{13}C-NMR spectrum of compound 2c

Table 4: The chemical shifts of carbon signals of the prepared compounds

Carbon atom	Chemical shift for 2b	Chemical shift for 2c	Assignment
C-1	123.69	122.58	Ar. Ring
C-2	161.55	167.15	Ar. Ring
C-3	113.33	117.51	Ar. Ring
C-4	136.08	132.55	Ar. Ring
C-5	144.87	144.80	Ar. Ring
C-6	127.37	127.56	Ar. Ring
C-7	172.33	172.33	COOH salicylic
C-8	155.63	136.07	Ar. Ring
C-9	130.69	130.29	Ar. Ring
C-10	119.61	118.87	Ar. Ring
C-11	148.56	152.25	Ar. Ring
C-12	117.52	119.60	Ar. Ring
C-13	129.58	130.69	Ar. Ring
C-14	-	171.72	COOH benzoic

3.4. Analgesic activity

The compound 2c was more active than other compounds in phase I, compound 2b was more active in phase II. So the *para* substitution compounds more active than *ortho* substitution compound in this research, as shown in Table 5 and Figure 9. The activity of compound 2b in phase II may be due to metabolize to active compound that inhibition of COX enzymes.

The compounds 2b and 2c can form hydrogen bonding with active site of COX enzyme more than other compound 2a. Compound 2c contain additional carboxyl group make his create propagate hydrogen bonding with peptide bond in the active site of COX-I and/or COX-II enzymes, possibly with COX-III enzyme.

Table 5: The hot plate test of the compounds

Time / Compd.	Latency time (sec.)		
	Zero time	**1:30 h**	**3:00 h**
2a	4 ± 1.4	8 ± 2.3*	10 ± 2.6*
2b	4 ± 2.1	9 ± 2.5*	7 ± 2.4
2c	3 ± 1.6	5 ± 1.9	11 ± 3.2*
Aspirin	2 ± 1.5	4 ± 1.7	6 ± 2.0

Note: the value ± SD, * = $p < 0.05$ evaluate according to aspirin In ANOVA t-test.

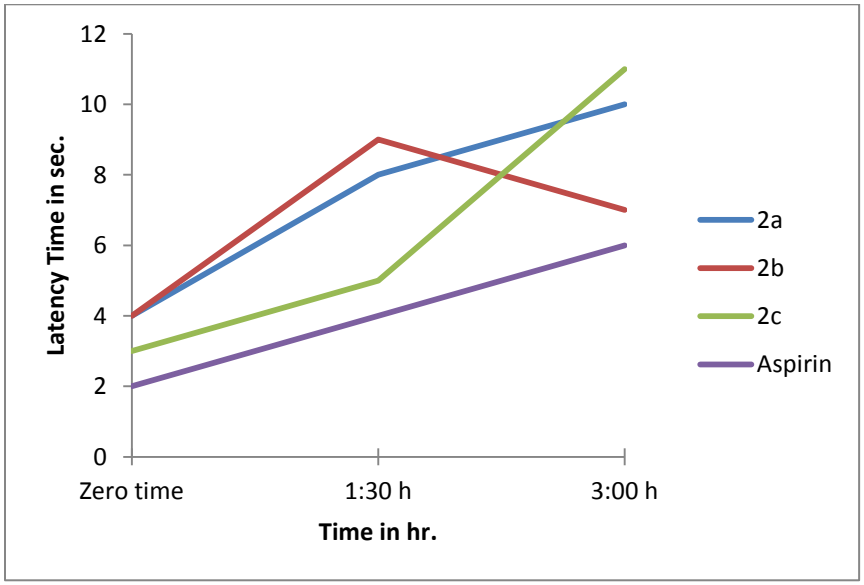


Figure 9: Hot plate test of compounds

4. Conclusion:

The prepared azo dyes compounds were prepared with high yield and purity. The compounds gave significant analgesic properties as compared with aspirin. The compound 2c gave the best resistant time for the mice at 3hrs which may referred to the present of two carboxylic acid groups in the compound.

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