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ISOLATION OF 3-(PHENYLAMINO) BENZOIC ACID FROM SEEDS OF RAPHANUS SATIVUS

Annotation: *The choloroformic extract of seeds of Raphanus sativus is separated and resulted in the isolation of one compound 3-(phenylamino)benzoic acid.*

The structure of the compound was detrmained by spectroscopic technique, and comparison of the NMR(1H-NMR,13C-NMR, DEPT(90º), DEPT(135º), HETCOR, HMBC), Ms, and IR data with the data found in the literature.

Keywords: *Raphanus sativus, 3-(phenylamino) benzoic acid, NMR.*

ИЗОЛЯЦИЯ 3-(ФЕНИЛАМИНО) БЕНЗОЙНОЙ КИСЛОТЫ ИЗ СЕМЕН РАТАНОВОГО САТИВУСА

Аннотация: *Хлороформный экстракт семян Raphanus sativus отделяется и приводит к выделению одного соединения 3-(фениламино) бензойной кислоты.*

Структуру соединения определяли с помощью спектроскопического метода и сравнения ЯМР) 1H-ЯМР, 13C-ЯМР, DEPT (90°), DEPT (135°), HETCOR, HMBC (, Ms и IR) с данными, найденными в литература.

Ключевые слова: *Raphanus sativus, 3- (фениламино) бензойная кислота, ЯМР.*

1. Introduction: Radish, Raphanus sativus Linn. (Brassicaceae family) is an annual herb, consumed as vegetable. Commonly known as Mooli. It is coarse, rough or glabrous. Leaves are lyrate, pinnate or pinnatifid. Flowers are large yellow, white or pale lilac, veined with purple, in long ebracteate racemes. Seeds are pendulous, globose; cotyledons conduplicate. Cultivated all over sub-continent up to 16,000 ft in temperate and warm countries [1, c.178]. It is well reputed in Unani System of Medicine, useful for urinary complaints and piles. Almost all parts of the plant including leaves seeds and roots are utilized in medicine. The fresh juices obtained from leaves are diuretic, laxative. Roots are used for urinary complaints and syphilitic disease; they are a reputed medicine for piles and gastrodynic pains. The seeds are expectorant, diuretic, laxative, carminative, antitussive and stomach tonic [2,c.300], Radish seeds also contain some glucosinolates and studies have proved useful in preventing cancer [3,c.302-304,4,c.208] and Recent studies have shown that derivatives of the compound 4-Methylthio-butanyl isolated from the seeds of radish plant anti-tumor properties [5,c.305-308].

Figure 1: seeds of Raphanus sativus Figure 2: Raphanus sativus

2. Materials and Methods:

2.1 Instrumentations:

Melting points were measured on an Electrothermal Entineering melting point apparatus / LTD / and are uncorrected.

MS, ^{1}H-NMR,^{13}C-NMR, and IR spectra were recorded on GC-MS-QP 2010 Shimadzu Bruker Ultra Shield 400MHz and Jasco FT-IR 410 respectively.

Rotational evaporator / Buchii /, analyzing preparative plates /TLC/ made of glass and aluminum, painted with Silica gel / Merck /, and solvents / Merck/.

2.2 Plant collection and extraction procedure:

Seeds of Raphanus sativus. were collected from zaidal area in Syria, in 2009, air-dried (500 g) and extracted twice with n-Hexane. Obtained extracts were combined and concentrated under low pressure, yielding 48 g of extract I. The residue from extract I was further extracted with CHCl3 and the obtained extract was then concentrated under vacuum resulting in 65 g of extract II.

5 g from extract II was loaded on column of silica gel (230 – 400 mesh, ASTM) and subjected to column chromatography (2×120 cm). The column was eluted successively with: n- hexane: chloroform (70:30, 600 ml), chloroform (400 ml), methanol: chloroform (5:95, 500 ml), and methanol: chloroform (10:90, 500 ml).

2.3 3-(phenylamino) benzoic acid:

A white solid was obtained from fraction (5gr) of extract II and purified on preparative TLC by using of CHCl3: MeOH (99.5: 0.5, Rf =0.37) mixture and recrystallized from methanol: chloroform mixture to give 3-(phenylamino)benzoic acid (39 mg). The compound is soluble in cold CHCl3 and in methanol and ethanol.

IR (KBr) cm-1 : 1263, 1591, 1657, 3040, 3338.

Mass: m/z (%) : M+ 213 (60 %), 195(100), 167(50), 139(13), 77(17),92(11).

^{1}H-NMR and ^{13}C-NMR (CDCl3) δ (ppm) see Table 1

3. Results and discussion:

3.1 Elucidation of structure of 3-(phenylamino) benzoic acid:

3-(phenylamino) benzoic acid was isolated from the concentrated chloroform extract of the air – dried leaves and flowers of the plant using silica gel column chromatography.

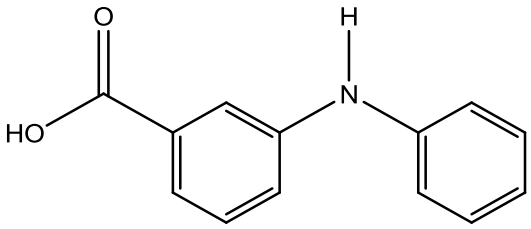

Figure 3: isolate compounds from Raphanus sativus

The determination of the structure of 3-(phenylamino) benzoic acid, was based on the usual spectral methods. Thus, the IR spectrum a broad band at 3040 cm^{-1} (O-H stretching), strong absorption band at 1657 cm^{-1} (C=O) and medium bands at 1263 cm^{-1} (C-N bending).

Moreover, the ^{13}C-NMR, exhibits 11 signals indicating the presence of at least 11 carbon atoms in the molecule. (Table 1, Figure 5)

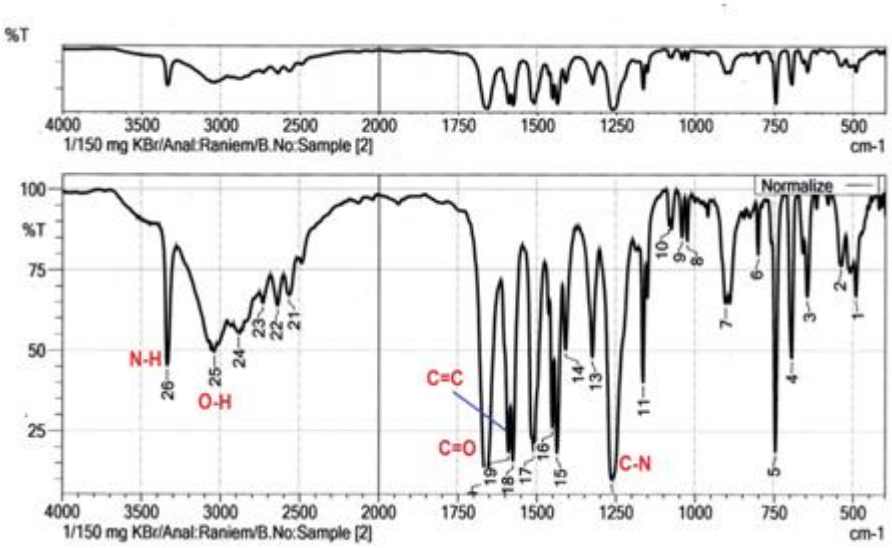

Figure 4: IR spectrum of 3-(phenylamino)benzoic acid

DEPT-135, and DEPT-90, however, show that these include 7 tertiary and 4 quaternary carbons. (Figure 6,7).

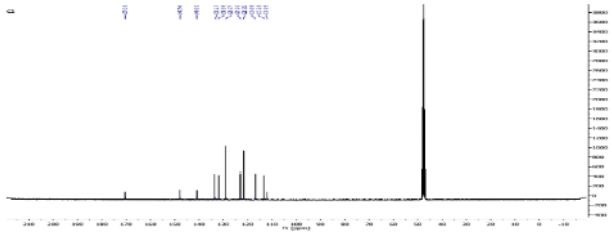

Figure 5: ^{13}C-NMR of 3-(phenylamino)benzoic acid in $CDCl_3$

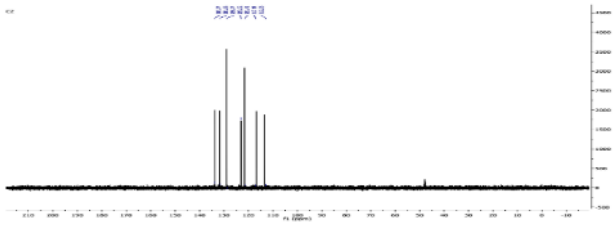

Figure 6: DEPT 135 of 3-(phenylamino)benzoic acid in $CDCl_3$

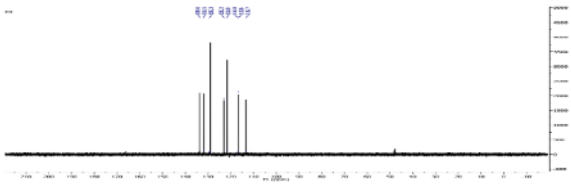

Figure 7: DEPT 90 of 3-(phenylamino)benzoic acid in $CDCl_3$

On the other hand, the mass spectrum of compound shows the molecular ion peak at m/z 213 corresponding to the correct molecular formula $C_{13}H_{11}O_2N$ (Figure 8).

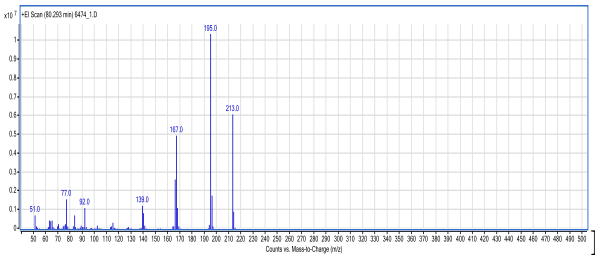

Figure 8: Mass spectrum of 3-(phenylamino)benzoic acid

The latter with 9 degrees of unsaturation, pointed to the two Aromatic ring and one double bond (C=O). Together with hydroxyl group. The spectrum of 13C-NMR Supports this conclusion.

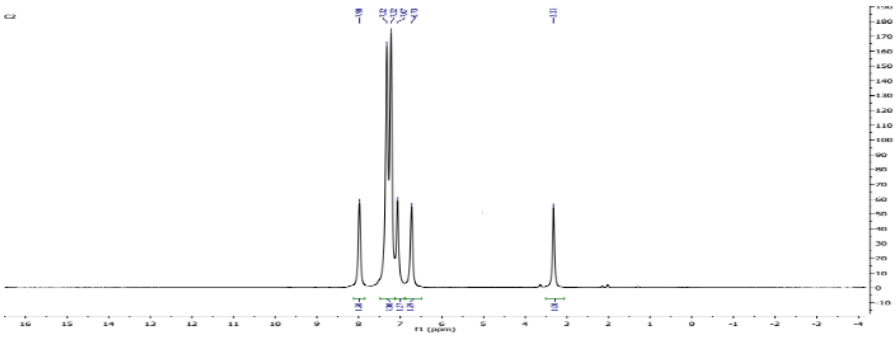

Figure 9: ^{1}H-NMR of 3-(phenylamino)benzoic acid in $CDCl_3$

As mentioned above, there is a absorption band at (3040 cm-1) in the IR spectrum of OH, as well as a absorption band at 1657 cm-1 for the CO group. In the NMR spectrum we note that there is a proton group at (8 ppm) For a single proton and not combined in the HETCOR spectrum with any carbon atom. This proton group is monoclonal and is not intertwined with any other proton if the large displacement of this group can be deduced from the proton group and we can write the following part:

From the ^{1}H-NMR spectrum (Figure 9), there is a proton group at (3.32 ppm) with a mono signal that is not intertwined with other proton groups. It is a single proton. Looking at the HETCOR spectrum, we observe that this group is not correlated with any carbon atom. Association NH (3338 cm^{-1})

In the spectrum of ^{1}H-^{1}H-COSY (Figure 12) we note that the proton group (3.32 ppm) of the nitrogen atom in the compound is not intertwined with any other proton group. This indicates that the carbon atoms adjacent to the nitrogen atom are quaternary carbons and since the compound contains only two rings So it is the nitrogen atom that binds the two aromatic rings.

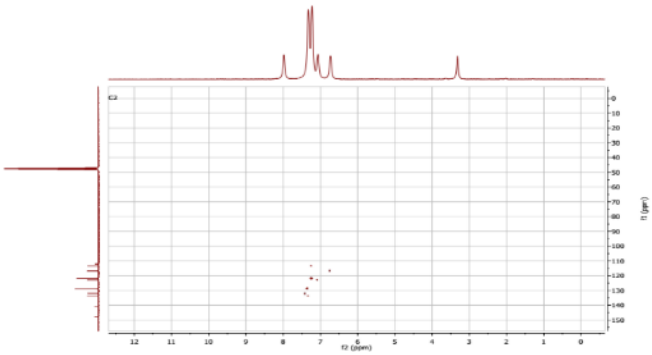

Figure 10: HETCOR of 3-(phenylamino)benzoic acid in $CDCl_3$

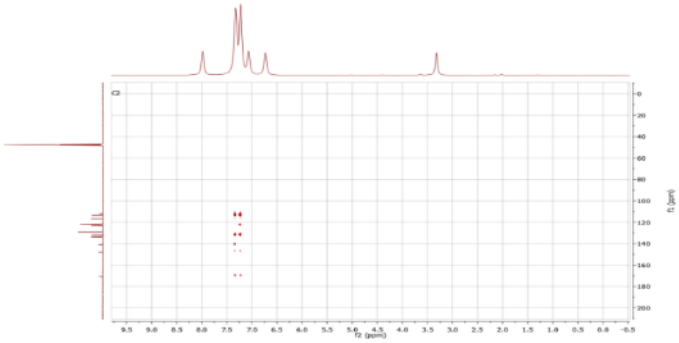

Figure 11: HMBC of 3-(phenylamino)benzoic acid in $CDCl_3$

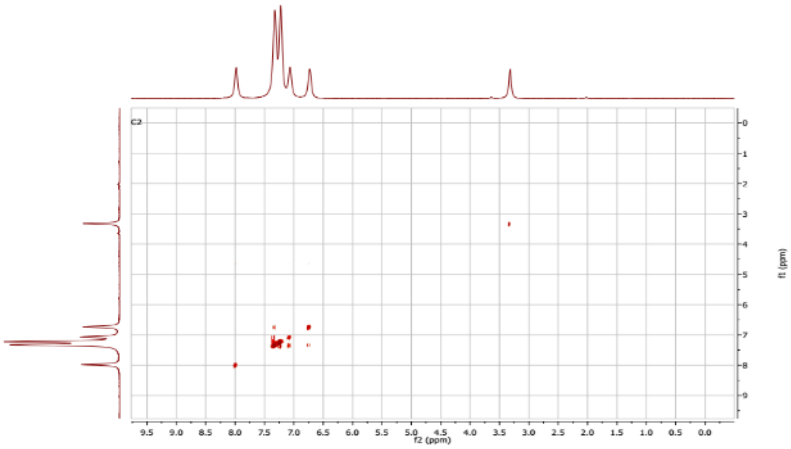

Figure 12: ^{1}H-^{1}H COSY of 3-(phenylamino)benzoic acid in $CDCl_3$

C	^{13}C δC (ppm)	DEPT (135-90)
1	111.95	C
2	113.26	CH
3	116.98	CH
4	121.75	CH
5	121.75	CH
6	123.02	CH
7	128.77	CH
8	128.77	CH
9	131.98	CH
10	133.27	C
11	140.92	C
12	147.74	C
13	170.36	C

Table 1:, ^{13}C-NMR and DEPT data of 3-(phenylamino)benzoic acid

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