Applied **B**iology in **S**aharan **A**reas *Vol. 1, N. 1, p. 8-12*
ISSN: 2571- 9823

Valorization of novel fatty acid isolated from *Citrullus Colocynthis* L. seed oil

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Abstract – One fatty acid was isolated from the petroleum ether extract of C.colocynthis seeds by column and thin layer chromatography. The composition of this acid was determined by gas chromatography. The elucidation of molecular structure was effectuated by spectral analysis and molecular modelling.

Keywords : Fatty acid, GCP, Analysis, molecular modelling

1. Introduction

Citrullus colocynthis L. is a perennial herbaceous vine indigenous to warm, dry regions of Africa and Asia and cultivated occasionally. The plant was well-known to the Greeks, Romans and Arabians physicians (Claus E.P. et al., 1970). It is an old remedy of moslem and Hindo Medecine and Familiar in Algeria under the name of " Hantal ". Alimachandani and al. (Alimachandri R.L. et al., 1949), Yankov and Hussein (Yankov L. & Hussein S.M, 1975), showed that the oil from the seeds of Citrullus colocynthis L. contains saturated hydrocarbons, saturated and unsaturated higher fatty acids and alcohols.

The aim of this work is to identify a novel fatty acid ($C_{22}H_{38}O_2$) isolated from the petroleum ether extract of seeds part of *Citrullus colocynthis* L.

2. Experimental

2.1 Materials

Citrullus colocynthis L. was collected from the Bechar region (Bidandou) in autumn 1998, and was shed dried at 25°C. A voucher specimen of the plant was identified and authenticated at the botany laboratory of Biology Institute. The dried plant was then separated into: roots, leaves, barks, stems, seeds and pulp. Then each part was ground by an electrical mill mesh and the powdered parts were kept separately in nylon bags in a deep freeze until the time of use.

- Thin-layer chromatography was performed on Silicagel 60 F_{254} Merck 0,25 mm thick.
- Developing system used was petroleum ether / Chloroform (9:1).
- Column chromatography was carried out on silica gel S Merck (60 F_{254}, 0,063-0,2 mm).

- Gas chromatography of the fatty ester was carried out on a GIRDEL gas chromatograph, column 2m , packed with DEGS on chromosorb W(60-80 meshes). Gas carrier nitrogen, rate of paper 5mm/mn, temperature programmed for 70-180°C with 3°C/mn. at 210°C isothermal injection conditions. Detection was made on a Flame Ionizing Detector.
- IR spectra was recorded on a PYE UNICAM SP3-200 spectrophotometer in a liquid layer (NaCl).
- ^{1}H.NMR-spectrums were recorded respectively on PERKIN-ELMER HITACHI-60Mz and BRUCKER AC-200MHz instruments in $CDCl_3$ solution in the presence of TMS as an internal standard. The signals are given in δ.
- ^{13}C-NMR spectrum was recorded on BRUCKER AC-200 MHz instrument in $CDCl_3$ solution in the presence of HMDS as an internal standard.
- Synthesis spectral was recorded on softword C13-SIMUL (13C.RMN: C.13-SIMUL. V.3.0, 1993).

2.2 Methods

2.2.1 Isolation of C. colocynthis seed oil

100 g of the powder seeds of Citrullus colocynthis L. was extracted by petroleum ether(40-60°C) for about 2 hrs. Following filtration, the solvent was distilled off. Yield: 16,7g of oiliness residue (Jeane-Bruneton; 1999).

2.2.2 Isolation of fatty acids

Into a 250 ml round-bottomed flask, fitted with a reflux condenser, place 3,40g of oiliness residue, Ethanol(15 ml), Water (15 ml) and Sodium hydroxide 3g. Reflux the mixture for about 45 min. Distill off the Ethanol under reduced pressure using a rotary evaporator. Extract the solution with 15 ml of diethyl ether. Acidulate the Organic layer with concentrate acid (HCl) then extract its with 20 ml of diethyl ether . Distill off the solvent. Yield: 3,24g of fatty acids residue (Gaston.J.Beaudoin et al,1991)..

2.2.3 Methylation of fatty acids

Into a 250 ml three-necked flask equipped with a drooping funnel, a sealed stirrer unit a double surface condenser, place 3g of fatty acids and 25 ml of methanol. Add slowly through the dropping funnel and with vigorous stirring a solution of concentrate sulfuric acid (1 ml). Reflux the mixture for about 2 hrs. Allow the reaction mixture to reach room temperature and to stand for 2 hours, cool the mixture and pour onto 300g of crushed ice . The aqueous layer was then extract with chloroform. Dried the Organic layer with anhydrous sodium sulfate. The solvent was distilled off. Yield: 1,66g of methyl esters residue (Gaston.J.Beaudoin et al,1991) (B.S. Furniss et al.,1978).

2.2.4 Chromatographic analysis of methyl esters extract.

The methyl esters extract (2g) was chromatographed on column 1150 mm long and 35mm in diameter, packed with 250 g of silicagel S, eluted subsequently with a mixture of petroleum ether-chloroform (9:1). A total of 10 fractions were collected and united into 2 fractions, one major(1,6g) and one minor(0,4g) fraction. One component was obtained in a pure state with the aid of preparative thin-layer chromatography.

3. Results and discussion

The major methyl ester is a transparent liquid which on thin layer chromatography gives one spot with Rf 0,60 (petroleum ether/chloroform 9:1).

The gas chromatogram of methyl esters of fatty acids shows the presence of one major methyl ester with retention time 8mn40s (Fig.1). The comparison of this retention time value with those given in tables revealed that this methyl ester possesses more 20 carbons (J. Willy Sons, 1968) (Hatam et al., 1990).

The IR-spectrum of major component showed bands at 2855; 2962,52; 2926; 1362,67; 1436,13; 1462,77and 1120,78cm^{-1}(CH_3 — and —CH_2—) and a band at 723,70cm^{-1} characteristic of straight carbon chain. Bands at 1743,37; 1171,40 and 1196,62cm^{-1} are characteristic of ester groups of aliphatic compounds with a straight carbon chain, while the bands at 1654 and 3009,23cm^{-1} revealed the presence of double bonds (R.N. Jones, 1959).

The ^{1}H.NMR 60 and 200 MHz-spectra showed a proton signal at 3,59 ppm(3H) assigned to methyl ester group. The ^{1}H.NMR 200 MHz-spectrum showed also a massif at 5,22-5,32 ppm typical of ethylinic group. In other wise integration bent gives a value of 40 protons (N.Bhacca, & P.Williams, 1964) (K.yamaguchi,1970).

The ^{13}C.NMR spectrum shows respectively three singlets at 13,78; 50,95 and 173,75ppm characteristic to carbon type **C**H_3; COO**C**H_3 and **C**OOCH_3. The spectrum gives also a massif characteristic to carbons hybridized on sp^2(**C**=**C**). The analysis of the ^{13}C.RMN data showed that the methyl ester contains twenty three carbons (sixteen carbons hybridized on sp^3 and seven carbons hybridized on sp^2 (H.Günther , 1996).

The combination of the results given below lead us to propose the following molecular formula for the methyl ester $C_{23}H_{40}O_2$.

In order to elucidate the developped structure of this ester, we used the spectral synthesis (simulation). This technic is based on construction a lot of developped molecular structure and comparison the values of experimental spectrum to theses of theoretical spectrum (**Table1**).

Table 1. Results of simulation study of methyl ester

δ ppm (Theoretical spectrum values)		δ ppm (Experimental spectrum values)	
1. 13,72	**13.** 33,40	**1.** 13,78	**13.** 31,33
2. 22,09	**14.** 33,47	**2.** 22,37	**14.** 31,74
3. 26,79	**15.** 33,47	**3.** 22,49	**15.** 33,75
4. 29,29	**16.** 50,70	**4.** 24,72	**16.** 50,95
5. 30,53	**17.** 127,76	**5.** 25,39	**17.** 127,68
6. 31,39	**18.** 127,76	**6.** 26,97	**18.** 127,81
7. 31,39	**19.** 128,08	**7.** 28,92	**19.** /
8. 32,95	**20.** 128,08	**8.** 28,97	**20.** 129,42
9. 33,09	**21.** 128,08	**9.** 29,16	**21.** 129,64
10. 33,09	**22.** 128,08	**10.** 29,29	**22.** 129,79
11. 33,09	**23.** 172,42	**11.** 29,39	**23.** 173,65
12. 33,09		**12.** 29,51	

The simulation study showed us that our methyl ester doesn't contain a ring and conjugaison between the three double bonds and the carbonyl group.

After comparison the δ(ppm) data of theoretical and experimental spectrums, we arrived to propose the fellow developped structure for the methyl ester of fatty acid (O.Ivancius et al., 1996) (O.Ivancius et al., 1997) (A.Panaye et al., 1993) (M.Carabedian & J.E.Dudois, 1998).

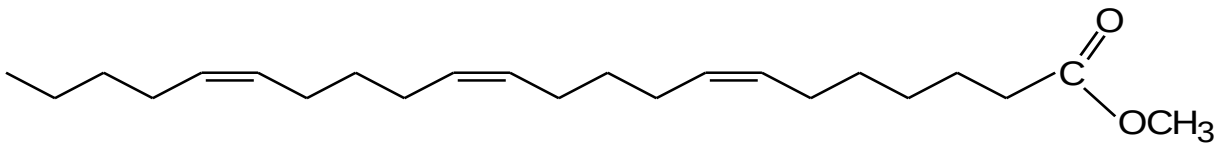

Figure 1. Proposed developped structure of methyl ester of fatty acid.

As conclusion , the *Citrullus colocynthis* is a rich plant on fatty acids. Extraction of oils from the seeds, their saponofication and esterification aloowed us to isolate a novel fatty unsaturated fatty acid which is elucidated *via* spectral analysis.

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