Synthesis and identification of some new amide alkaloids compounds from the extraction of cauliflower

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Abstract:

The present study includes synthesis of new amide alkaloid derivatives from cauliflower alkaloids extracted by petroleum ether . the *cauliflower* is from species called (*Brassiceoleracea*). The resulting compound(*botrytis alkaloid*) react with ethanol alcohol to produced (ester alkaloid)then react with different amino acid (Phenyl alanine ,Tryptophan, Tyrosine ,Histidine ,glutamine & asparagine) to give amid alkaloid derivates. The chemical structure of these compounds were identification by spectral techniques.

Keywords: alkaloid, *brassiceoleracea*, botrytis ,ester ,Amino acid .

1. Introduction

The Alkaloids are one of the important organic compounds ,that contain Nitrogen atom in their heterocyclic composition and have basic properities with special physiological actions,so some of them are stimulatants, e.g (caffeine) ,others are anaelgesics,e.g (cocaine & morphine) ,anxiolytics ,anaesthetics,e.g (codeine)¹. There is no boundary between alkaloids and other nitrogen-containing compounds. Compounds as peptides, amino acid , nucleotides, proteins, , nucleic acid, antibiotics , and amines are often not called alkaloids. The Alkaloids have a low-molecular-weight and since it has a heterocyclic ring containing a nitrogen atom so it is typically an alkaline. Alkaloids have numerous pharmacological effects on vertebrates². The Mechanism of Action of Alkaloids vary considerably. It may cause toxicity by enzymatic alterations affecting inhibition of DNA synthesis and repair by intercalating with nucleic acids, or by affecting the neurological system. the ability to act as antibacterial agents vary among alkaloid classes ³. For decades, the medicine use the extract of (afyon) as first drug in treating insomnia. Alkaloids are found in plants as salts of organic acids⁴. There are many different plants that contain variable types of alkaloids ,like (Cauliflowers) which are one of vegetables in the species *Brassiceoleracea⁴*,and contain sinaxalen alkaloid which are important in medicine this compound including sulforaphane and indol-3-carboinol which appears to work as anti estrogen⁵. In plants, alkaloids has the ability to act as defence compounds efficient against pathogens causing their toxicity by Fast perception of aggressors and efficient signal transduction producing alkaloid accumulation leading to successful plant protection. In general , toxic effects depend on dosage, time of exposure, and individual characteristics as sensitivity, developmental stage, and site of action⁶.

2. Materials and Instruments
All chemical used were supplied by Merck and CDH, BDH company. FT-IR spectra were recorded by using Fourier transformation infrared (Shimadzu-8400S) in Japan and (H\textsuperscript{1}NMR-spectra & C\textsuperscript{13}NMR-spectra) were recorded by (Bruker,400 MKZ) with DMSO-d6, melting point were determined by Electro Thermal Melting Point, UK and were uncorrected.

2.1 Alkaloids extraction from dietary plants (Cauliflower): 4

Cauliflower (80g) was mixed with distilled water (300mL). The mixture was shaken well 6h filtered it, then HCL (5mL) is added to the filtrate. The filtrate was shaken with a mixture of ethanol / petroleum ether (30 mL:60 mL), then it was left for 48h. The mixture was separated by separation funnel, alkaloid salts are in the aqueous liquid, (1g) added to it then filtrate mixed with (NaHCO\textsubscript{3}) the free alkaloid is precipitated and re-crystallized.

2.2 Prepare of ester alkaloid from compound (1) of Botrytis alkaloid: 7,8

By dissolving (1.05 gm, 0.01 mol) of alkaloid in ethanol (50 mL) was added sulphuric acid (1 mL) to room temperature. The reaction mixture was warmed and stirred for 24 hours, Then dried over CaCl\textsubscript{2} anhydrous MgSO\textsubscript{4} and concentrated in vacuo to afford the compound 2 as an off-white solid (4.3 g, 96%).

2.3 Synthesis Amides alkaloid compound (3-8): 9,10

To a solution of esters alkaloid alcohol (2.5 mL, 0.01 mol) in ethanol (50 mL) was added to (2 mL, 0.01 mol) of primary amine (Phenyl alanine, Tyrosine, Histidin, Tryptophan, Glutamine and Asparagine) respectively at room temperature. The reaction a mixture was warmed to 110°C and stirred for 20 hours by refluxed, The precipitate was filtered and re-crystallized to gave amides alkaloids derivatives of compound (3-8).

Figure 1. Synthesis of ester alkaloid compound (2)
3. Result and discussion

The chemical structure of (Botrytis alkaloid) contains carboxylic acid group which is used as starting material in synthesis of ester alkaloid \(^{11}\), in this work, the compounds (3-8) are prepared from diverse of amino acid to produce amid alkaloid derivative \(^{12,13}\) like compounds (3-8). All synthesized compounds (1-8) have been identified by (spectroscopic methods) FT-IR, \(^{1}H\)NMR, \(^{13}C\)NMR.

3.1 (FT.IR) spectrum

Presented an absorption band at 3080 cm\(^{-1}\) due to (OH)hydroxyl group of carboxylic acid, absorption band at 1693 cm\(^{-1}\) due to (CO) carbonyl group in compound 1, the spectra of compound 2, showed demise of absorption band due to (OH) of carboxylic acid appearance band at 2939-2831 cm\(^{-1}\) due to ethyl group of ester \(^{14}\). The spectra of compound 3-6 showed appear bands at 1678-1689 cm\(^{-1}\) due to (CO) carbonyl group of amide and 3360-3273 cm\(^{-1}\) due to (NH) of amide., The spectra of compound 7 and 8 showed appear of two absorption bands , of the (asym and sym) stretching vibration of (NH\(_2\)) group of glutamine and asparagines at 3466-3396 cm\(^{-1}\), the absorption band 1668-1660 cm\(^{-1}\) due to (CO) amide group respectively \(^{15}\), in these compound of amino acid and other data bands in table 1 and figures 1-8.

Table 1. FT.IR-data of alkaloid derivatives (1-8)
| Comp.No | FT-IR (cm⁻¹)                                                                 |
|---------|------------------------------------------------------------------------------|
| 1       | 3408(O-H acid), 3080(N-H), 3080(H-aromatic), 2926 and 2881(C-H aliphatic), (C=O acid), 1568(C=N of pyrimidine), 1514(C=C aromatic). |
| 2       | 3279(N-H), 2939-2831(C-H aliphatic), 1731(C=O of easter alkaloid), 1604(C=N of pyrimidine), 1573(C=C aromatic). |
| 3       | 3425(OH acid), 3358(N-H), 3039(H-aromatic), 2937 and 2872(C-H aliphatic), 1692(C=O of amino acid), 1680(C=O of amid), 1605(C=N of pyrimidine), 1581(C=C aromatic). |
| 4       | 3433(O-H acid), 3273(N-H of amid), 3047(C-H aromatic), 2931 and 2873(C-H aliphatic), 1700(C=O acid), 1689(C=O amid), 1654(C=N of pyrimidine), 1589(C=C aromatic). |
| 5       | 3427(O-H acid), 3360(N-H amid), 3039(C-H aromatic), 2939 and 2816(C-H aliphatic) of amid (1679), 1681(C=O acid), 1679(C=O amid), 1579(C=N pyrimidine), 1494(C=C aromatic). |
| 6       | 3365(O-H acid), 3309(N-H), 3036(C-H aromatic), 2937 and 2868(C-H aliphatic), 1719(C=O acid), 1678(C=O amid), 1583(C=N of pyrimidine), 1489(C=C aromatic). |
| 7       | 3492(O-H acid), 3466 and 3369(NH₂), 3230(N-H amid), 3043(C-H aromatic), 3001 and 2924(C-H aliphatic), 1701(C=O acid), 1668(C=O of CONH₂), 1627(C=O amid), 1595(C=N), 1492(C=C aromatic). |
| 8       | 3555(O-H acid), 3450 and 3373(NH₂), 3255(N-H amid), 3036(C-H aromatic), 2937 and 2872(C-H aliphatic), 1710(C=O acid), 1660(C=O of CONH₂), 1595(C=O amid), 1541(C=N), 1492(C=C aromatic). |
Figure 3. Infrared spectrum of the compound (1)

Figure 4. Infrared spectrum of the compound (2)
Figure 5. Infrared spectrum of the compound (5)

Figure 6. Infrared spectrum of the compound (6)
3.2 $^1$HNMR- Spectrum $^{14,15,16}$.

Showed signal for proton at δ10.170 ppm due to (OH) hydroxyl group of carboxylic acid in compound 1 but disappearance this signal in compound 2 while appeared signal for proton at δ1.1-2.5 ppm due to ethyl group easter alkaloid in this compound and showed signal proton of (NH-CO) amides group appeared in compounds 3-8 at δ 8.92-7.95 ppm which appear single at 6.6, 5.7 ppm respectively due to (NH2) primary amine in compound 7 and 8 other signal shown in table 2 and figures 1 and 8.

Table 2. $^1$HNMR-data (δ ppm, DMSO-d6) of amide alkaloids
Comp.No. $^1$HNMR

1 $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm: 10.17 (s, 1H C=O-OH), 7.08 – 6.70 (m, ph-H), 2.50 (s, 1H, N-H ), 1.20-1.18 (s, 2H,CH$_2$).

2 $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm: 7.41 – 6.56 (m, ph-H ), 2.50 (s, 1H,N- H ), 2.07-1.17 (s, 2H,CH$_2$).

3 $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm: 10.73 (s, 1H C=O-OH),8.48 (s, 1H,C=O-NH), 7.95 – 7.01 (m, aromatic-H), 3.77 (m, 1H, N-H indol ring ),3.04( s 1H ,CH),1.19 ( d 2H, CH$_3$).

4 $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm: 10.47 (s, 1H C=O-OH),9.26 (s, 1H,OH hydroxyl group),8.925(s,1H ,C=O-NH), 8.89 – 7.10 (m, aromatic-H), 3.45 (s, 1H, N-H indol ring ),3.45( s 1H ,CH), 1.21 ( t, 2H, CH$_3$).

5 $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm: 10.28 (s, 1H C=O-OH), 8.48 (s,1H ,C=O-NH), 8.29 – 7.74 (m, aromatic-H), 4.48-3.84 (s, 1H, N-H indol ring ),3.01( s 1H ,CH),1.19 ( t, 2H, CH$_3$).

6 $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm: 10.19 (s, 1H C=O-OH), 8.46 (s,1H ,C=O-NH), 7.77 – 6.95 (m, aromatic-H), 3.51 (s, 1H, N-H indol ring ),2.89( s 1H ,CH),2.50 ( s, 2H, CH$_3$).

7 $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm: 10.36 (s, 1H C=O-OH), 8.03 (s,1H ,C=O-NH), 8.01 – 6.64 (m, aromatic-H), 6.64( s,2H, NH$_2$), 3.05 (s, 1H, N-H indol ring ),1.19 ( s 2H ,CH$_3$),1.16 ( s, 2H, CH$_3$).

8 $^1$H NMR (400 MHz, DMSO-$d_6$) δ ppm: 10.73 (s, 1H C=O-OH), 7.95 (s,1H ,C=O-NH), 7.93 – 6.57 (m, aromatic-H), 6.55-5.77 ( s,2H, NH$_2$ ), 3.48 (s, 1H, N-H indol ring ),1.19 ( s 2H ,CH$_3$).

Figure 9. $^1$H-NMR spectrum of the compound (1)
Figure 10. $^1$H-NMR spectrum of the compound (2)

Figure 11. $^1$H-NMR spectrum of the compound (4)
Figure 12. \(^1\)H-NMR spectrum of the compound (5)

Figure 13. \(^1\)H-NMR spectrum of the compound (7)

3.3 \(^13\)C-NMR-Spectrum

Showed data of some compounds peaks indicated to important groups in these compounds, table three and figures.
Table 3. $^{13}$CNMR-data (δ ppm, DMSO-d$_6$) of some amid derivatives$^{[15,16,17]}$

| Comp.No | $^{13}$CNMR-data |
|---------|------------------|
| 1       | (C=Ar) at 130-139, (C=O) of carboxylic acid at 170.3, (CH$_2$ and CH) of heterocyclic at 35.5-39.7, (C=N) at 150.9, (C=C) at 140.0, (N-C-N) carbon of heteroaromatic at 140.5. |
| 3       | (C=N) at 155., (C=Ar) at 114-146., (CH$_2$ and CH) at 23.6-26.9., (C=C) at 149., (CO) of carboxylic acid at 180., (C=O) carbon of amide at 165, (NCN) carbon of hetero at 148. |
| 6       | (C=N) at 141.7-140.0., (C=Ar) at 113-130.4., (CH$_2$ and CH) at 21.4-30.7., (CO) of carboxylic acid at 174., (CO) carbon of amide 163., (NCN) carbon of hetero at 130.4. |
| 7       | (C=N) at 145.8., (C=Ar) at 128-140., (CH$_2$ and CH) at 20.9-22.8., (CO) of carboxylic acid at 172.6., (CO) carbon of amide 166.4&163.5., (NCN) carbon of hetero at 145.3. |

Figure 14. $^{13}$C-NMR spectrum of the compound (1)
Figure 15. $^{13}$C-NMR spectrum of the compound (3)

Figure 16. $^{13}$C-NMR spectrum of the compound (7)

Table 4. Physical properties of synthesized compounds

| NO | M. F       | M. P   | Rf  | Color     | %yield |
|----|------------|--------|-----|-----------|--------|
| 1  | C$_{12}$H$_{11}$SN$_3$O$_2$ | 105-107 | 0.7 | White     | 87     |
| 2  | C$_{18}$H$_{15}$SN$_3$O$_2$ | gummy   | 0.8 | off-white | 96     |
| 3  | C$_{24}$H$_{25}$SN$_4$O$_4$ | 156-158 | 0.65 | Red       | 85     |
| 4  | C$_{24}$H$_{25}$SN$_4$O$_5$ | 222-224 | 0.83 | Yellow    | 76     |
| 5  | C$_{24}$H$_{25}$SN$_4$O$_4$ | 233-235 | 0.94 | Orange    | 76     |
| 6  | C$_{21}$H$_{24}$SN$_6$O$_4$ | 217-219 | 0.85 | Yellow    | 81     |
| 7  | C$_{19}$H$_{22}$SN$_5$O$_5$ | 204-206 | 0.73 | Brown     | 83     |
| 8  | C$_{26}$H$_{26}$SN$_5$O$_4$ | 277-279 | 0.78 | Yellow    | 83     |
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