Abstract

3-nitropropionic acid (3-NPA) is a widespread nitroaliphatic toxin extracted from a variety of fungi and plants, 3-NPA was extracted from four species of Leguminosae grown in the field south west of Libya i.e. Hippocrepis cyclocarpa, Hippocrepis bicontorta, Astragalus hauaransis and Scorpiurus muricatus, the dried above-ground plant parts were extracted with acetone and purified by silica gel column chromatography. From the final fractions the residues were yield 3-NPA, and its structure was confirmed by Thin Layer Chromatography (TLC), Nuclear Magnetic Resonance (NMR) and Mass Spectroscopy (MS). Beside the 3-NPA, an aromatic nitrocompound was also detected in the extract of Astragalus hauaransis.

Keywords: 3-NPA, Leguminosae, TLC, NMR, MS

1. Introduction

A variety of higher plants are known that are capable synthesizing the nitrocompounds such as 3-nitropropionic acid (3-NPA) and 3-nitropropanol (3NPOH) and their derivatives (Gold & Brodman, 1991). It had been found in a large number of angiosperm species, representing four families, the Fabaceae, Malpighiaceae, Corynocarpaceae, and Violaceae (Gustine, 1979).

The occurrence of 3-NPA has been reported in 1920 (Gorter, 1920). Nitrocompounds have been reported in 450 species of genus Astragalus, 60 species of genus Indigofera and in 17 species of genus Lotus (Williams, 1982; Benn et al, 1992 and Salem et al, 1995). The levels of nitrocompounds found in plants vary greatly between species and are influenced to a certain extent by environmental conditions. It is also higher in leaves than in roots and stems (Salem, 1996). The concentration of nitrocompounds change according to the stage of development and are also affected by storage of plant materials (Majak & Wikeem, 1986 and Majak et al, 1988). Nitropropionic acid was also identified in some fungi such as Aspergillus flavus, A. oryzae, A. wentii and Pencillium atroventum (Wei et al, 1994) and prokaryotic organisms such as Streptomyces species. 3-NPA was also isolated from the arthropod beetle Chrysomelid tremulae which appeared to be associated with defensive glands (Randoux et al, 1991).

In general the biosynthesis of nitrocompounds and their esterification to glucose moieties are rare phenomena in plant kingdom (Candlish et al, 1969). It had been reported that both of carbon skeleton and the amino group of L-aspartic acid were incorporated into 3-NPA by Pencillium atroventum and the amino group was directly oxidized in situ (Baxter et al, 1985).

2. Materials and Methods

Plant samples were collected from the field during the spring season in 2006, aerial parts of the plant material dried at room temperature and kept in plastic bags. 20 grams were taken and extracted with 250 ml acetone for 72 hours, and filtered through Whatman filter paper. Shoots were washed once by 20 ml acetone, the extract
concentrated by rotary evaporator to about 8 ml. 3-NPA was eluted from silica gel column (55x4 cm) using 100 ml of 40-65% of ethyl acetate and 1% formic acid in chloroform, fractions were collected and concentrated by rotary evaporator to thickness and redissolved in small volume of acetone. Elutes were characterized by Thin Layer Chromatography (TLC) (Majak & Bose, 1974), nuclear magnetic resonance (NMR) and Mass spectroscopy (MS).

3. Results and Discussion

TLC of an acetone extract of the plant samples revealed the presence of 3-nitropropionic acid which gave a positive reaction for C-nitro compounds with diazotized p-nitroaniline reagent, it is \( R_f \) comparing to the authentic sample for A. hauaransis, H. bicontorta, H. cyclocarpa and S. muricatus were (0.29, 0.35, 0.51 and 0.31) respectively. These \( R_f \) values were differ from that reported by Williams (1985) and Salem et al (1995). This difference might be due to the existence of other nitrocompounds, working conditions and the use of an old thin layer papers. However the \( R_f \) values were compatible with the \( R_f \) values of the standard 3-NPA.

3-NPA was extracted from A. hauaransis as described in materials and methods. \(^1\)H- NMR Spectrum of 3-NPA (Figure 1). Thin layer chromatography shows the compound was in one fraction of the elution (60% of ethyl acetate: 1% formic acid in chloroform)

The 300 MHz NMR Spectrum of the isolated 3-NPA in (d4-MeCO2) showed at \( \delta 3.05 \) (t, \( j = 5.8 \) Hz -O2CCH2CH2NO2), at \( \delta 3.02 \) (t, \( j = 5.9\) O2CCH2CH2NO2), at \( \delta 4.78 \) (t, \( j = 5.85 \) Hz -OCH2CH2), \( \delta 4.47 \) (t, \( j = 5.85 \) Hz -OCH2CH2), OH was not detected. This result is consistent with the results reported by (Pailer & Nowotny, 1958 and Moyer et al, 1979).

An aromatic nitrocompound (Figure 4) was also found in the same extract, the coupling constant of H-NMR (d$_2$-MeCO$_2$) showed at \( \delta 7.46 \) (dd, \( j = 3.45 \) Hz) At \( \delta 8.3 \) (dd, \( j = 3.48 \) Hz) the two mentioned compounds were reported for the first time in this species according to Hersenhorn et al (1993).

3-NPA was also extracted from H. bicontorta (Figure 5). In 55% ethyl acetate in 1% formic acid in chloroform H-NMR spectrum, showed that the coupling constant in (d$_2$-MeCO$_2$) Showed at \( \delta 3.05 \) (t, \( j = 5.79 \) Hz -O2CCH2CH2NO2), at \( \delta 3.02 \) (t, \( j = 5.9\) O2CCH2CH2NO2), at \( \delta 4.78 \) (t, \( j = 5.9\) Hz -OCH2CH2), OH was not detected. This outcome is...
consistent with the results reported by Pailer & Nowotny (1958); Stermitz et al (1975) and Moyer et al (1979). The existence of 3-NPA is reported for the first time in this plant.

3-NPA was also extracted from *H. cyclocarpa*. The fraction 60% of ethyl acetate: 1% formic acid in chloroform, (Figure 6) H-NMR spectrum, showed that the coupling constant in (d$_4$-MeCO$_2$) at δ 3.05 (t, $j = 5.79$ Hz -O$_2$CCH$_2$CH$_2$NO$_2$), at δ 3.02 (t, $j = 5.9$ -O$_2$CCH$_2$CH$_2$NO$_2$), at δ 4.78 (t, $j = 5.79$ Hz -OCCH$_2$CH$_2$NO$_2$), at δ 4.47 (t, $j = 5.9$ Hz -OCH$_2$CH$_2$), OH was not detected. This result is consistent with the results reported by Pailer & Nowotny (1958), Moyer et al (1979); Jabber, 2008 and Hipkin et al (2004).

3-NPA was also found in fraction of 55% ethyl acetate: 1% formic acid in chloroform of *S. muricatus* (Figure 8) the H-NMR Spectrum showed that the coupling constant in (d$_4$-MeCO$_2$) at δ 3.05 (t, $j = 5.79$ Hz-O$_2$CCH$_2$CH$_2$NO$_2$), at δ 3.02 (t, $j = 5.9$ -O$_2$CCH$_2$CH$_2$NO$_2$),

4. Conclusion

It was reported that 3-NPA was extracted from many species of family Leguminosae, in this study we confirm
the existence and extraction of 3-NPA in four species, native in Libya, an aromatic nitro compound has also been isolated from *Astragalus hauaransis* which need deep investigation.

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**References**


