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Full Length Research Paper

Isolation and characterization of dipeptide derivative and phytosterol from *Capparis tomentosa* Lam

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In this study, chemical investigation of the constituents of the roots of *Capparis tomentosa* was carried out. *C. tomentosa* is a medicinal plant used extensively for the treatment of various diseases. It is also known to be poisonous. The crude petroleum ether extract of the roots of this plant was subjected to column chromatography for separation. Progress of separation was monitored by thin layer chromatography. The isolated compounds were recrystallized for further purification. The structures of these compounds were elucidated by comparison of their spectroscopic data from IR, ^{1}HNMR, ^{13}CNMR and MS analysis with that of authentic samples. The isolated compounds were identified as 24-ethylcholestan-5-en-3-ol a phytosterol and a dipeptide derivative, N-benzoylphenylalanylaninol acetate. This is the first time these compounds have been isolated from this plant.

Key words: *Capparis tomentosa*, dipeptide derivative, N-benzoylphenylalanylaninol acetate, 24-ethylcholestan-5-en-3-ol, phytosterol.

INTRODUCTION

Capparis tomentosa Lam is a shrub which belongs to the family *Capparidaceae* which has four genera. *C. tomentosa* which belongs to the genus *Capparis* grows in the savanna forest of Western, Eastern and Southern Africa (Irvine, 1961; Marganet, 1965). This plant is found widely in local pharmacopoeias throughout Africa. The plant is used extensively for the treatment of variety of ailments, including mental disorder, snake bites, chest pains, impotency and barrenness (Abbiw, 1990; Burkill, 1965; Watt and Breyer-Braindwijk, 1962). It is also used as a cure for wounds and leprosy (Watt and Breyer-Braindwijk, 1962; Steenkamp, et al., 2004). The plant is also known to be very poisonous to livestock and man. There had been reports of several fatal cases of poisoning from the use of this plant as medicine (Ahmed et al., 1981; White, 1983; Ahmed et al., 1993).

Judging from the extensive uses to which *C. tomentosa* is put and the fact that the plant is poisonous, it can be seen that this plant merits scientific investigations. The aim of this study is to isolate and purified as many compounds as possible from the roots of *C. tomentosa* Lam and determine their structures.

The genus *Capparis* is large, however only a limited phytochemical and pharmacological work has been carried out on it. Compounds that have been isolated and characterized from the genus *Capparis* include flavornoids, fatty acids, lipids, alkaloids and glucosinolates. Several species of the genus *Capparis* have been reported to contain glucosinolates. Glucosinolates are a uniform class of glucosides with a sulphur atom in its structure and are know to cause goiter. In other studies, glucocapparin was found to be present in the leaves of *Capparis spinosa*, *Capparis flexuosa* and *Capparis linearis* as well as in the seeds of *Capparis ovata* and *Capparis angulata* (Gmelin and Kjaer, 1970; Kjaer, 1955; Cornforth and Henry, 1952). Further investigation of other *Capparis* species resulted in the isolation and detection of glucocoiberin, glicocapparin, sinigrin, glucobrassicin, 4-oxo-heptylglucosinolateand neogucograssicin (Cornforth and Henry, 1952).

In view of the reported toxicity of this plant to camel, the fruits were examined and L-stachydrine (1) was isolated as periodide and analyzed as the HCl salt from ethanol extract of the seeds pulp (Cornforth and Henry, 1952). In

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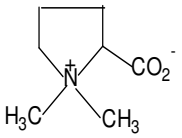
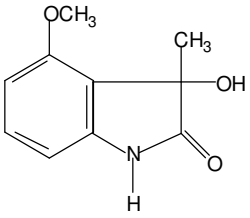


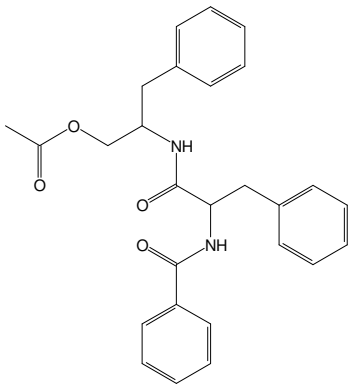
(1)

L-Stachydrine



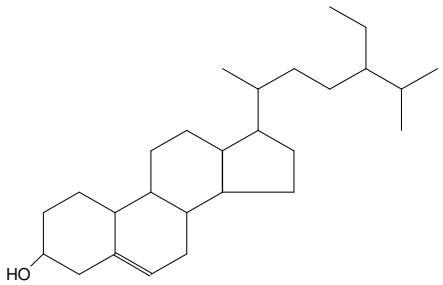
(2)

3-Hydroxy-3-methyl-4-methoxyoxindole



[3]

N-Benzoylphenylalanylaninol acetate



[4]

24-Ethylcholestan-5-en-3-ol

Figure. 1. Compounds isolated from *Capparis tomentosa*.

another study Dekker et al. (1993) isolated from the roots and oxindole (2) which was identified as 3-hydroxy-3-methyl-4-methoxyoxindole from the roots have been reported (Figure 1).

As part of a continuing study of the constituent of this plant, structural elucidation of a dipeptide derivative (3) and a phytosterol (4) are reported (Figure 1).

MATERIALS AND METHODS

Plant material

The roots of *C. tomentosa* were dug from some shrubs on the Accra plains around the University of Ghana farms. The roots were cut into smaller pieces and dried in the shade for three weeks and pulverized. Voucher specimen is kept at the Ghana National Herbarium, Department of Botany, University of Ghana, Legon.

General method

Separation and purification of the various components of the crude extract were mainly done by column chromatography (CC) and recrystallization. Silica gel 60 (Fluka) was the main adsorbent used for the chromatography. Solvent commonly used include petroleum ether, ethylacetate (EtOAc) and chloroform. Qualitative Thin layer Chromatography (TLC) was used to monitor the progress of the column and also to ascertain the purity of the components. TLC were run on aluminum and glass plates precoated with silica gel 60 F_{254} (Merck) with a thickness of 0.2 mm. Visualization of spots on TLC was by Ultra Violet (UV) light, Iodine vapour and Anisaldehyde spray reagent (Stahl and Kaltenmech, 1961). Melting points of pure components were determined on a Gallenkemp Melting Point Apparatus. Infrared (IR) spectra were recorded on a Shimadzu IR-408 spectrophotometer. IR samples were run in mull in nujol. Proton (^{1}H) and ^{13}C Nuclear Magnetic Resonance (NMR) spectra were recorded in Germany on Brucker 250 spectrophotometer at a frequency of 360 MHz for ^{1}H and 90 MHz for ^{13}C. These spectra were obtained on solutions of samples in deuterated chloroform ($CDCl_3$) with tetramethylsaline (TMS) as internal reference. Mass spectral (MS) were run on a Finningan mass spectrometer, by electron impact (EI) at 70ev.

Extraction

Pulverized roots (2.4 kg) were extracted with 12 liters of petroleum ether (60 -80°C) in a soxlet apparatus for 48 h. The solvent was removed under reduced pressure to obtain a dark brown oily substance (12.2 g) with Rotary Evaporator. The extract gave 8 spots on TLC developed in a petroleum ether/EtOAc (4:1) solvent system.

Investigation of extract

The crude petroleum ether extract was taken up in a minimum amount of petroleum ether and introduced onto a column packed with silica gel. Elution was started with petroleum ether followed by petroleum ether/EtOAc (95:5) mixture. The percentage of the

EtOAc was gradually increased until 100% EtOAc was finally used. About 15 cm^3 portions of the eluent were collected and monitored by TLC. Based on the TLC result, the eluents were combined into 6 fractions (F_1-F_6). Rechromatography of F_4 as described above gave 5 fractions (C_1-C_5). Fraction C_2 yielded 24-ethylcholestan-5-en-3-ol (4) (Figure 1) which crystallized as white needles from petroleum ether.

Fraction F_5 was dissolved in minimum amount of chloroform and introduced onto a column. Elution was done with petroleum ether followed by 5% petroleum ether/chloroform. The percentage of chloroform was increased till pure chloroform was used. The eluent were combined into 4 main fractions (A_1-A_4). The solid obtained from A_3 was washed with activated charcoal in chloroform. It was then recrystallized in petrol to obtain a light brown granular solid. This solid was identified as N-benzoylphenylalanylaninol acetate (3) (Figure 1).

RESULTS AND DISCUSSION

Separation of the petroleum ether (60 – 80°C) extract of the roots of this plant by chromatography on silica gel resulted in the isolation of N-benzoylphenylalanylaninol acetate (3) which was identified by comparison of its spectral properties with literature data (Kyoto et al., 1991). The compound had a molecular ion peak (m/z) at 444 for $C_{27}H_{28}O_4N_2$ by high resolution mass spectrometry. The IR spectrum indicated the presence of an ester carbonyl group (1735 cm^{-1}) and amide groups (3410, 1670 and 1645 cm^{-1}) which presence were also supported by two proton signals (δ 6.72 and 5.95) in the ^1H NMR spectrum and by two amide carbon signals (δ 167 and 170.5) in the ^{13}C spectrum. The ^{13}C NMR spectrum gave 27 signals due to two amide carbons, acetyl carbons (δ 20.77 and 170.5), 18 aromatic carbons, three methylene carbons (δ 37.5, 38.5 and 64.6) and two methine carbons (δ 49.5 and 55). The presence of unsubstituted benzoyl and benzyl groups were confirmed by the intense fragments ions m/z 105 (100%) and m/z 91 (13%), respectively. The substitution pattern of each group was confirmed by the fragment peaks at m/z 224 (36%) and 252 (36%) resulting from bond cleavage at either side of the central carbonyl group. The above spectral data confirmed the structural of (3) as shown below.

The phytosterol, 24-ethylcholestan-5-en-3-ol (4) was identified by comparison of its IR, ^1H-NMR and ^{13}C-NMR and MS spectra with reference spectral of authentic samples. The IR spectrum gave absorption band for C-O stretch of a secondary alcohol at 1070 cm^{-1} and a C-H bending of a CH_3 at 1460 and 1380 cm^{-1}. A broad peak at 3450 cm^{-1} indicates the presence of –OH in the molecule. The signal at δ 3.5 ppm in the ^1H-NMR can be assigned to the hydroxylic proton. The fact that the molecule is highly saturated can be seen from the ^1H-NMR and ^{13}C-NMR spectra, where all the signals occur at the sp^3 hybridized portion of the spectra. In the ^{13}C-NMR, the peaks at δ 128 and 138 ppm were due to two sp^2 hybridized carbons. The peak at δ 71 ppm is assigned to the carbon bearing the hydroxyl group. The mass spectrum showed a molecular ion peak at m/z 414 (89%) calculated for $C_{29}H_{50}O$ and a base peak at m/z 55 (100%). The fragmentation pattern in the Ms was quite complex.

Spectral data (N-benzoylphenylalanylaninol acetate)

Light brown granules from petrol, mp. 174-176. Anisaldehyde: yellow. IR V_{max} cm^{-1} 3410, 1735, 1670, 1665. ^1H NMR δ2.02 (3H, s), δ3.07 (1H, dd) δ3.22 (1H, dd), 3.84 1H, dd), 4.35, (1H, m) 4.78 (1H, ddd), 6.03 (1H, d) 6.73 (1H, d), 7.05-7.21 (5H, m), 7.21-7.30 (5H, m), 7.42 (2H, t) 7.51 (1H, d), 7.70 (2H, dd). EIMS (rel int %) m/z 444.205 (M^+), 353 (16), 323 (17), 313 (5), 311 (26), 209 (9) 224 (36), 176 (9), 172 (18), 147 (5), 106 (100), 91 (13), 77 (13) and 133 (14).

Spectral data (24-ethylcholestan-5-en-3-ol)

White crystals from petrol, mp 132-133°C. IR V_{max} cm^{-1} 3450, 2900, 1460, 1380, 1070. 1H-NMR δ3.5ppm (OH) all other signals occurred in the sp^3 hybrid portion of the spectrum ^{13}C NMR δ138, 128 (C=C), 71 (-OH) all the other signals occur at the sp^3 hybrid portion of the spectrum. EIMS (rel int %) 414$(M)^+$ (89), (calculated for $C_{29}H_{50}O$) 329 (35), 321 (21), 273 (32), 199 (16), 213 (48), 255 (70), 281 (35), 241 (7), 133 (14), 121 (35), 55 (100).

Conclusion

From the chemical investigation conducted on *C. tomentosa* Lam, two compounds were isolated, charac-terized and identified as N-benzoylphenylalanylaninol acetate and 24-ethylcholestan-5-en-3-ol (Figure 1). This is the first time these compounds have been established from this plant.

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