Pakistan Journal of Social Science, 3 (5): 751-754, 2005
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Studies on the Constituents of *Ficus capensis* (Thunb)

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Abstract: The first report for isolation and identification of 4, 4, 24-trimelhyl-cholesta-8-en-3-B-ol, mixture of campesterol, stigmasterol and. β-sitosterol, stigmasterol 3-B-o'glucoside and 4, 5, 7-trihydroxy flavan-3-ol. In addition to xantholoxin, β-amyrin and α-amyrin from n-hexane and ethyl acetate fractions of ethanol extract of *Ficus capensis* (Thunb) leaves. Identification of these compounds has been established by spectral evidences (UV, IR, MS, ^{1}H and ^{13}C-NMR). *Ficus capensis* (Thunb) Family Moraceae is an evergreen tree widely distributed in tropics. Various parts of the tree has been reported to possess medicinal properties. Both leaves and roots have been used internally as tonic, anti-rheumatic, to reduce fever and for treatment of tuberculosis while externally. They were used for leprosy, leucodermia and swollen fects.[3,4] Previously we reported the isolation and characterization of different constituents from *Ficus benjemina* and *Ficus elastica*. Some of these compounds showed interesting biological activities. In the course of our studies on the constituents of different *Ficus* plants we examined the constituents of *Ficus capensis* (Thunb). This study deals with the isolation and identification of flavanol, coumarins, steroids and triterpenes from the leaves of the title plant.

Key words: *Ficus capensis*, 24-trimelhyl-cholesta-8-en-3-B-ol, β-sitosterol

INTRODUCTION

Melting points were uncorrected and determined by Koffler hot stage microscope. IR spectra were taken in KBr using Unicam SP 1025 spectrometer. UV spectra were run in MeOH using Sp 1750 spectrometer ^{1}H-NMR were determined by XL-300 (varian) and Bruker 250 spectrometers. ^{13}C-NMR were recorded on XL-300 (Varian) Bruker 250 and Jcol FI (Fx 60). TMS was used as internal standard and chemical shifts are given in ppm. Mass spectra were recorded on Finnegan quadrupole GCMS-QP 5050 spectrometer at 70 eV and Perkin-Elmer sigma 3B for GC/MS/EL.

Silica gel GF_{254} (Merck) was used for TLC and silica gel (E. Merk) was used for column chromatography. H_2SO_4 50% was used for visualizing sterols and triterpenes, thymol / H_2SO_4 for sugars, UV light (254, 366 nm) for coumarins and flavanol.

The following solvent systems were used:

Syst. I: hexane-ethyl acetate (9:1)
Syst. II: hexane-ethyl acetate (8:2)
Syst. Ill: chloroform-methanol (9:1)
Syst. IV: chloroform-methanol (8:2)
Syst. V: ethyl acetate-hexane (8:2)
Syst. VI: n-butanol-acetone-formic acid-water (60:17:8:15)

MATERAIALS AND METHODS

Plant material: The leaves of *Ficus capensis* (Thunb) were collected on December 2002 from tress cultivated in Juba University Campus and identified by Prof. Dr. Ekhlass Abd-El-bari, Department of Botany, Faculty of Science, University of Khartoum. The collected material was air-dried reduced to powder No. 40 and kept for extraction.

Extraction and isolation: The air dried powdered leaves (2.0 kg) of *Ficus capensis* (Thunb) were exhaustively extracted with ethanol 80% and concentrated under reduced pressure, the concentrate was subjected to solvent fractionation using n-hexane and ethyl acetate. The obtained fractions were separately concentrated and screened by thin layer chromatography and paper chromatography for different constituents.

A hcxane Fraction: Hexane fraction (20.0 g.) was fractionated on silica gel column (800.0 g. 7 x 210 cm). Elution was started with n-hexane and then with hexane / ethyl acetate gradient, fractions 150 ml each were collected, concentrated and screened by thin layer chromatography (silica gel G. syst. I, II and III). Similar fractions were combined concentrated and subjected for crystallization. Five pure compounds were obtained and labeled (1-5). Where compounds 1 and 2 were obtained from the group eluted with hexane-5% ethyl acetate, compound 3 was eluted with 7% ethyl acetate in hexane, compound 4 with 12% ethyl acetate in hexane and compound 5 with 20% ethyl acetate in hexane.

β-Ethyl acetate Fraction: Ethyl acetate fraction (15.0 g.) was fractionated on silica gel column (600.0 g, 5 x 180 cm). Elution was started with chloroform followed by chloroform/methanol gradient. Fractions 150 mL each were

collected, concentrated and examined by thin layer chromatography (silica gel G, syst. IV and V). Fractions eluted with chloroform 3% methanol, were evaporated and purified by preparative thin layer chromatography (solvent syst. IV) to give compound 6. While fractions eluted with chloroform-10% methanol were concentrated, passed over short alumina column and eluted with chloroform 2% methanol where compound 7 was obtained.

Acid hydrolysis of compound 5: 10.0 mg of the isolated glycoside was dissolved in 5 ml methanol to which 5 mL of 5% hydrochloric acid were added. The mixture was refluxed for 3 hours on a boiling water bath, cooled, the aglycone was extracted with chloroform, purified and subjected to thin layer chromatography. Sugar was LC (Silica gel G, syst. VI).

Compound 3: White crystals (chloroform) 300 mg, m.p. 285-87°C: IR (γ max cm^{-1}): 3380, 2940, 1650, 1460, 1390 and 820. ^{1}H-NMR (400 MH_Z, MeOH) at δ: 3.82 (lH, m, H-3 α), 1.29 (6H, s, 29-30-Me), 1.18 (3H, d, J=6 Hz, 24-Me), 1.16 (3H, d, J=6 Hz, 21-Me), 1.06 (6H, d, J=6 Hz, 26-.27-Me). 1.0 (3H, s, 19-Me). and 0.94 (3H, s, 18-Me). MS: M+at m/z 428, 413-(M^+-15), 410 (M^+-18), 395 (M^+-33), 301 [M^+-s.c (C_9H_{19}), 259 (M^+-(s.c.+42), 207, 189, 165, 121, 95, 55 and 43. ^{13}C-NMR (25 MHz, MeOH) at δ: 36.6 (C-1), 32.5 (C-2), 71.4 (C-3), 38.5 (C-4), 61.9 (C-5), 18:0 (C-6), 30.2 (C-7), 135.4 (C-8), 135.2 (C-9), 37.5 (C-10), 35.0 (C-11), 39.9 (C-12), 43.2 (C-13), 50.0 (C-14), 30.9 (C-15), 31.9 (C-16), 53.5 (C-17), 12.5 (C-18), 18.6 (C-19), 39.5 (C-20), 20.2 (C-21), 35.9 (C-22), 32.2 (C-23), 42.3 (C-24), 32.2 (C-25), 18.9 (C-26), 20.3 (C-27), 17.0 (C-28), 16.6 (C-29), 28.3 (C-30).

Compound 4: White flakes (methanol) 500 mg. IR (γ max cm^{-1}): 3340, 2940, 1650, 1460, 1380, 1080 and 820. MS (m/z. rel. int.) component A: M^+at 400 (100), 385 (36), 382 (48), 367 (29), 315 (38), 289 (51), 273 (27), 213 (33), 105 (34) and 43 (74). Component B: M+at 412 (86), 394 (11), 369 (22), 351 (25), 300 (43), 271 (51), 255 (71), 213, 161, 139. Component C: M+at 414 (100), 396 (43), 381 (26), 329 (34), 303 (46), 273 (22), 255 (25), 231 (20), 213 (30), 161, 159 and 55.

Compound 5: White amorphous powder (acetone) 100 mg, m.p. 233-35°C. IR (γ max cm^1): 3400, 2940, 1650, 1450, 1370, 1610, 1070 and 1020. ^{1}H-NMR (400 MHz, MeOH) at: δ: 5.35 (1H, dd, J=7.5, 2.5 Hz, H-6), 5.2-5.3 (2H, m, H-22, H-23), 5.07 (lH, d, J=7.5 Hz, H-1), 4.42-4.0 (6H, m, sugar protons), 3.95 (IH, m, H-3α), 0.98 (3H, d, J=6.5 Hz, Me-2l), 0.90 (3H, d, J=6.5 Hz, Me-26), 0.85 (3H, t, Me-29), 0.85 (3H, s, Me-l9), 0.84 (3H, d, J=6.5 Hz, Me-27), 0.64 (3H, s, Me-l8). Ms: M^+at m/z 412, 394, 369, 351, 300, 271 and 255.

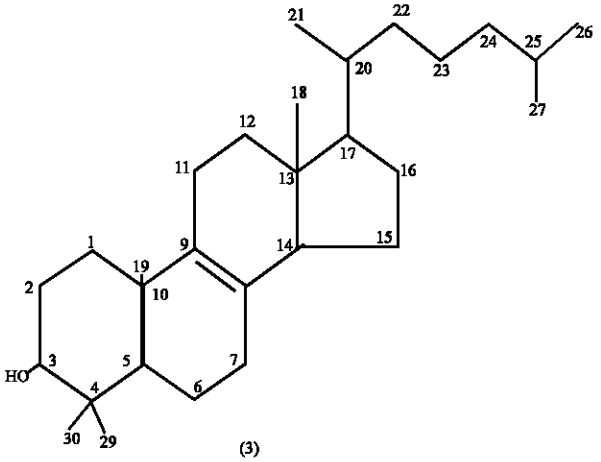
Compound 6: Yellowish-white crystals (methanol) 30 mg, m.p. 145-47°C. IR (γ max cm^{-1}): 3100, 1720, 1700, 1610, 1570, 1530. UV λ^{MeOH} max, nm: 270, 294 (sh), 302;+NaOH: no change. ^{1}H-NMR (400 MHz, $CDCl_3$) at δ: 7.68 (lH, d, J=9.5 Hz, H-4), 7.6 (1H, d, J=2 Hz, H-2); 7.25 (lH, s, H-5), 6.74 (lH, d, J=2 Hz, H-3), 6.22 (1H, d, J=9.5 Hz, H-3), 4.15 (3H, s, OCH_3). ^{13}C-NMR ($CDCl_3$, 25 MHz) at δ: 160.4 (C-2), 146.5 (C-7), l44.3 (C-4), 142.9 (C-8α), 132.9 (C-8), 126.1 (C-6), 116.4 (C-14), 114.6 (C-3), 113.6 (C-5), 106.7 (C-12), 61.2 (OCH_3). Ms (m/z, rel.int.) M+at 216 (100), 201 (M^+-CH_3, 30), 188 (M^+-CO, 15), 173 (57), 145 (30), 89 (90), 63 (85) and 51.

Compound 7: Buff needles (methanol) 250 mg, m.p. 237-38°C. IR (γ max cm^{-1}): 3300-3500, 1625, 1520, 1470, 1220, 1140, 820, 800. UV λ^{MeOH}max: 228, 276. ^{1}H-NMR (400 MHz, $CDCl_3$) at δ: 6.43 (2H, dd, J=2 Hz and 8 Hz, H-2' and H-6'.), 5.92 (2H, dd, J=2 Hz and 8 Hz, H-3' and H-5'), 5.1, 5.07 (IH, d, J=2 Hz, H-6 and H-8), 4.2 (IH, m, H-3), 4.0 (lH, d, J=6 Hz, H-2), 2.0 (2H, dd, J=16 Hz and 9 Hz, H-4). ^{13}C-NMR ($CDCl_3$, 25 MH_Z) at δ: 157.9 (C-7), 157.8 (C-5'), 157.5 (C-8a), 157.3 (C-4'), 131.6 (C-1'), 129.2 (C-2' and C-6'), 115.8 (C-3' and C-5'), 100.1 (C-4a), 96.5 (C-8), 96.1 (C-6), 79.9 (C-2), 67.3 (C-3) and 29.3 (C-4). MS (m/z, rel. int.) M+at: 274 (55), 256 (M^+-18.2), 139 (100), 136 (48), 107 (70), 77, 69 and 65.

RESULTS AND DISCUSSION

The air-dried powdered leaves of *Ficus capensis* (Thunb) was extracted with ethanol 80% and the concentrated extract was fractionated with n-hexane and ethyl acetate. Each extract was concentrated and chromatographed on silica gel column the n-hexane fraction afforded five pure compounds. Compounds 1 and 2 were identified as β and α-amyrin by direct comparison of melting point, co-chromatography, acetate melting point and superimosable IR spectra with authentic samples. Compound 3 gave reddish violet colour with Liebermann-Burchard test[1-3] indicating a steroid like skeleton. IR spectrum showed OH function (3380 cm^{-1}) and unsaturation function at (1650, 820 cm^{-1}). ^{1}H-NMR revealed the presence of four tertiary methyl groups (singlets), four secondary ones (doublets) and a multiplet at δ: 3.8 corresponds to a proton attached to a hydroxyl carbon. The mass spectrum showed molecular ion peak at m/z 428, fragments at m/z 301, 127 indicated C_9H_{19} side chain without unsaturation which was also confirmed by absence of olefmic proton signals in ^{1}H-NMR. Cleavage of ring C gave a peak at m/z 207 suggesting the dimethyl substitution at C-4, while a fragment at m/z 189 (207-H_2O) confirmed the presence of OH group, in addition to mass

fragmens at m/z 413 (M^+-CH_3), 410 (M^+-H_2O), 395 [(M^+-(CH_3+H_2O)], 259 [M^+-(S.C+42)]. The mass fragment at m/z 284 of composition $C_{21}H_{32}$ indicated a steroid skeleton of four stable rings resulting from the loss of side chain and a hydroxyl group. ^{13}C-NMR exhibited 30 signals suggested the presence of at least 30 carbon atoms, signals at 135.4, 135.2 were attributed to C-8 = C-9[4] while a signal at δ: 71.4 corresponding to C-3 OH[5].



(3)

Based on ^{1}H, ^{13}C-NMR, IR and mass spectral features, 4,4,24-trimethyl cholest-8-en-3-B-ol was assigned for compound 3.

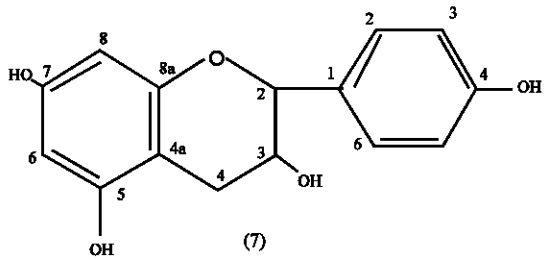
Compound 4 gave bluish-green ring with Salkowisk's test, indicating its steroid nature[3]. Its IR spectrum showed hydroxy function at (3340 cm^{-1}) unsaturation function at (1650, 820 cm^{-1}) and aliphatic C-H stretching vibrations at 2940 cm^{-1}.

Thin layer chromatography of the prepared acetate using silica gel G impregnated with 10% $AgNO_3$ and solvent system [n-hexane-$CHCl_3$-HOAC (75:25:0.5)], revealed the presence of three spots. GC/MS analysis of compound 4 showed three peaks with retention indices 1617, 1686 and 1753. The three components were identified by MS as campesterol, stigmasterol and β-sitosterol. Stigmasterol was recognized as the most abundant sterol.

Compound 5 gave positive test for steroids[3] and gave positive Molish's test indicating its glycosidic nature. Its IR spectrum showed wide band at 3400 cm^{-1} for hydroxyl groups, unsaturation bands at 1645 and 830 cm^{-1} and bands at 1610, 1020 for pyranose sugars. Its ^{1}H-NMR spectrum showed characteristic pattern for unsaturated sterols with characteristic signals at δ: 5.35 for H-6 and at δ: 5.2-5.3 for H-22 and H-23. It also revealed a multiple! at δ: 3.9 which was attributed to H-3α an anomeric proton at 5.07 (d, J=5.7 Hz for B-linked glucosides) in addition to two tertiary methyl groups (as singlets) and four secondary ones (as doublets). Acid hydrolysis of compound 5 gave sugar fraction which was identified by thin layer chromatography as glucose and the aglycone was identified as stigmasterol by comparing its mass spectral data with a reported one, m.p and co-chromatography with authentic samplee. Based on the spectroscopic finding discussed above, compound 5 was identified as stigmasterol-3-B-O-glucoside.

Compound 6 showed characteristic blue fluorescence under UV light which was intensified by exposure to ammonia vapour. The appearance of UV bands at 270, 249 (sh) 302 nm. IR bands at 1720-1700 cm^{-1} (θ-lactone) and at 1620, 1570 cm^{-1} (aromatic C-C stretching) suggested a furano coumarin skeleton. ^{1}H, ^{13}C-NMR and mass spectral data of compound 6 compared favorably with those previously reported for xanthotoxin[10-12].

Compound 7 gave blue fluorescence under UV light and an orange colour with anisaldehyde-sulphuric acid, indicating that it may be a flavan derivative[13]. Its UV spectrum in methanol showed only two absorption bands at 228 and 276 nm which was not affected by shifting agents while IR exhibited strong hydroxy absorption at 3300-3500 cm^{-1} but no carbonyl absorption was observed. ^{1}H-NMR spectrum exhibited two pairs of (dd) two protons each, (with J=2,8 Hz) characteristic for protons of para substituted benzene ring system.[14] It also revealed two doublets with meta coupling characteristic for H-6 and H-8. The up-field methylene protons at 2.0 and 29.3 in ^{1}H and ^{13}C-NMR spectrum were attributed to C-4 protons of a flavan system[15,16]. ^{13} C-NMR spectrum showed 15 signals suggesting the presence of at least 15 carbon atoms, the signals at δ: 131.5, 129.2, 115.8, 157.3 were attributed to ring C carbons comparison with related structures[17,18]. While signals at δ: 96.9, 95.9, 157.9 and 157.8 were corresponded to ring A carbons. The mass spectrum of the investigated compound showed molecular ion peak at m/z 274 with a base peak at m/z 139 which confirmed the hydroxy substitution at C-5 and C-7 in addition to characteristic and significant peaks at m/z 136 and 107[18]. Based on the above discussed spectroscopic data compound 7 could be identified as 4',



(7)

5, 7-trihydroxy flavan-3-ol and according to the available

literature it could be considered the first report for its isolation and identification from *Ficus capensis*.

ACKNOWLEDGMENT

The author wish to thank to all colleuges for their interest during the course of this investigation in University of Juba, University of Khartoum (Sudan) for facilities and to the Prof. Dr. M. B. Rahmani, University Putra Malaysia for facilitating spectral analysis.

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