Bisbenzylisoquinoline Alkaloids from *Epinetrum villosum* (Menispermaceae)

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Summary: A phytochemical investigation of the stem bark of *Epinetrum villosum* (Menispermaceae) resulted in the isolation of six bisbenzylisoquinoline alkaloids: cycleanine, cycleanine *N*-oxide, norcycleanine, cocsoline, cocsuline, and a new alkaloid cocsuline N-oxide.

Introduction

Epinetrum villosum (Exell) Troupin (*Albertisia Villosa* Farm), a woody cylimber of Triclisieae tribe of the Menispermaceae family is found in secondary forests in Xerophytic woods on hills in the coastal area of Zaire and Angola. The plant is used natively for the treatment of pains and mental strain [1,2,3]. In 1958, Denoil [4] and in 1971, Bouquet and Cave [5] reported the isolation of cycleanine, norcycleanine and isochondodendrine from this plant. We now report in addition to these alkaloids, the isolation of cycleanine *N*-oxide, cocsoline and a new alkaloid cocsuline *N*-oxide. These alkaloids have been identified by means of spectral data (UV, MS, ^{1}H-NMR) TLC and colour reactions [5-22].

Results and Discussion

The five known alkaloids: cycleanine, cycleanine *N*-oxide, norcycleanine, cocsoline and cocsuline were identified by direct comparison of UV; IR; TLC; mp; ^{1}H-NMR and colour reactions with authentic samples [5-14]. The new alkaloid proposed to be cocsuline N-oxide gave mp. 210°-212° C; $[\alpha]_D^{25}$ + 264° (C = 0.3, $CHCl_3$); λ_{max} (CH_3OH) nm. 278, 291, 305 (similar to that of cocsuline), [13,14]; ν_{max} (KBr) similar to that of cocsuline except for an additional peak at 1500 cm^{-1} which is indicative of N-O stretch of *N*-oxide [21,22]. The mass spectrum m/z 578 (M^+, 95%) corresponding to $C_{35}H_{34}N_2O_6$, (16 mass units higher than that of cocsuline $C_{35}H_{34}N_2O_5$), and a prominent peak m/z 562 (M^+ - 16, 45%) due to the loss of an oxygen atom suggests the presence of an *N*-oxide function [15,16,23]. The ^{1}H-NMR spectrum was similar to that of cocsuline except that one of the *N*-methyl signal was shifted down field (δ 3.36 as opposed to δ 2.56) suggesting again the presence of *N*-oxide function [17,18].

We therefore, report that the new alkaloid is an *N*-oxide of cosuline and has the possible stereo structure 1 (Fig. 1). Further substantiation was done by, reduction of 1 with 10% H_2SO_3 which afforded cocsuline, identical by a direct comparison (UV, IR, TLC properties and chromogenesis) with an authentic sample of cocsuline obtained from *Synclisia scabrida* [12], and oxidation of the authentic sample of cocsuline obtained from [12], with 15% H_2O_2 to get cocsuline *N*-oxide which showed TLC and colour reactions identical to alkaloid 1. These observations confirmed that the new alkaloid 1 is *N*-oxide of cocsuline. It is unlikely that the alkaloid is an artifact formed during the isolation procedure as it can be detected by TLC in the test extracts of *Epinetrum villosum* stem bark.

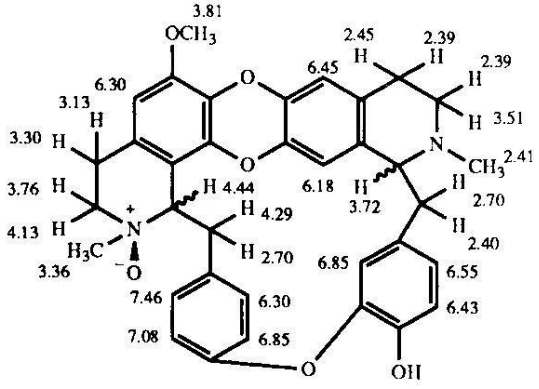

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Fig. 1: Possible structure of cocsuline N-oxide

Experimental

Plant material

The plant material was obtained from Mr. Breyne Heiman (39A rue van Page, 1080,

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Brussele), Hebarium I.N.E.R.A., Kinshasa, Zaire. A voucher specimen had been deposited at the Department of Chemistry, Bendel State University, Ekpoma.

Extraction, chromatography and detection

The bark was removed from the stems of Epinetrum villosum, dried and ground to a coarse powder. The pulverised stem bark (1 Kg) was extracted with 90% MeOH (3 dm^3) in a glass percolator to yield a syrupy residue (5 g) after removal of solvent in vacuo. The residue was dissolved in 300 cm^3 of 3% HCl (pH-2). After basification with 25% NH_4OH (pH-8), the solution was extracted with $CHCl_3$ (3 x 150 cm^3). The $CHCl_3$ extracts were combined, washed with H_2O, dried over anhydrous Na_2SO_4 and evaporated to dryness in vacuo, after filtration, to yield a non-quaternary alkaloid fraction (2.0 g).

The nonquaternary alkaloid fraction was dissolved in $CHCl_3$ (50 cm^3) and chromatographed over a silica gel (type 60, 100 mesh ASTM) column (3.5 x 40 cm) packed in $CHCl_3$. Elution was begun with $CHCl_3$ and the polarity was increased gradually by addition of MeOH (10%, 15% and 25%). Fraction of 500 cm^3, were collected and monitored by TLC. Eventual elution with $CHCl_3$ - MeOH (4:1) afforded a fraction (1.0 g) containing bisbenzyliso-quinoline alkaloids with two or three diphenyl ether linkages. Final separation was achieved by means of repeated preparative TLC, on 20 x 20 cm, precoated glass plates with 0.5 mm thick layer of silica gel 60 F_{254} (Merck) in the solvent systems:$CHCl_3$ - MeOH - conc. NH_4OH (10:2:0.1) and EtOAc-$(Me)_2CHOH$ 25% NH_4OH (9:7:2). The second solvent system was used to determine the R_f values of the separated alkaloids.

The alkaloidal zones were detected under UV_{254}, UV_{366} and iodoplatinate reagent. Two colour reactions were performed by means of (i) 0.2 M $FeCl_3$ in 35% $HClO_4$ and (ii) a new colour "colour ring test", to distinguish the bisbenzylisoquinolines with two and three ether linkages. A developed chromatograpm after spraying with H_2PtC_{l6} - KI (1:10), dried at 25°C and again sprayed with HNO_3 - HCl - H_2SO_4 (1:1:1) and heated at 100°C for exact 5 min. A bisbenzylisoquinoline with two diphenyl ether linkages (cycleanine) gave a reddish brown spot surrounded by a yellow, grey and violet rings, while the one with three diphenyl ether linkages (cocsuline) gave a reddish spot surrounded by a violet ring.

Identification

UV spectra were recorded in methanol on a Perkin Elmer model 137UV instrument. The IR spectra were taken as KBr disc on a Perkin Elmer model 257 grating infrared spectrophotometer. Optical rotations were registered with a Perkin Elmer model 141 polarimeter (Na-590 nm). Melting points (uncorrected) were determined using a hot-stage microscope. ^{1}H-NMR spectra were obtained in $CDCl_3$ on a Hitachi Perkin Elmer model R-24, high resolution spectrometer.

Cycleanine

^{1}H-NMR: 2.60 (2 x N-CH_3), 3.40 (2 x O-CH_3), 3.82 (2 x O-CH_3) 4.31 (d, J=10-Hz, 2H), 5.81 (dd, J=8.5 Hz, J=2.7Hz, 2H), 7.1 (dd, J=8.5 Hz, J=2.0 Hz, 2H), (ArH), R_f 0.75; $[\alpha]_D^{25}$ - 18.2° (C = 0.30, MeOH), UV: 275, 285 nm. Colour reaction with $FeCl_3$ reagent gave an intense purple colour in hot air.

The data is identical with those for cycleanine [6-9].

Cycleanine N-oxide

^{1}H-NMR: 2.60 (N-CH_3), 3.32 (N'-CH_3), 3.42 (2 x O-CH_3, 3.84 (2 x O-CH_3), 4.31 (d, J=10Hz, 2H), 5.81 (dd, J = 8.5 Hz, J = 2.7 Hz, 2H), 6.27 (dd, J = 8.5 Hz, J = 2.0 Hz, 2H) 6.58 (dd, J = 8.5Hz, J = 2.7 Hz, 2H), 7.1 (dd, J=8.5, J=2.0Hz, 2H) (ArH). Rf 0.37:$[\alpha]_D^{25}$ - 7.6° (C=0.30, MeOH). UV, 275, 285 nm. Colour reaction with $FeCl_3$ reagent gave an intense purple colour to hot air.

The data is identical with those for cycloeanine *N*-oxide [12].

Norcycleanine

^{1}H-NMR: 2.6 (2 x N-CH_3), 3.40 (O-CH_3), 3.81 (O-CH_3), 3.84 (O-CH_3) 4.31 (d, J=10 Hz, 2H), 5.79 (bd, J=8.5 Hz, 2H), 6.25 (d, J = 8.5 Hz, 3H), 6.50 (s, 1H), 6.56 (s, 1H), 6.50 - 6.66 (m, 2H), 6.97 - 7.12 (m, 2H) (ArH). Rf 0.65 $[\alpha]_D^{25}$ + 23.5° (C=0.08, MeOH), UV: 275, 285 nm. Colour reaction with $FeCl_3$ reagent gave a brownish purple colour in hot air.

The data is identical with those for norcycleanine [7,10,11].

Cocsoline

^{1}H-NMR: 2.60 (N-CH_3), 3.85 (O-CH_3), 6.19 (s, 1H), 6.33 (s, 1H), 6.54 (d, J=2.7 Hz, 2H), 6.62 (s, 1H), 6.76-7.22 (m, 4H), 7.46 (dd, J = 8Hz, J = 2.7 Hz, 1H), (ArH). R_f 0.57; $[\alpha]_D^{25}$ + 198° (C = 0.08, $CHCl_3$), UV: 275, 285, 302 nm. Colour reaction with $FeCl_3$ reagent gave a greenish black colour in hot air.

The data is identical with those for cocsuline [13,14].

Cocsuline

^{1}H-NMR 2.43 (N-CH_3), 2.56 (N-CH_3), 3.81 (O-CH_3), 3.76 (s, 1H), 4.13 (s, 1H), 6.18 (s, 1H), 6.30 (s, 1H), 6.45 (s, 1H), 6.55 (d, J = 2.7Hz, 2H), 6.85 (s, 1H), 6.93 (dd, J = 8.0Hz, J = 2.7 Hz, 2H), 7.08 (bm, 1H), 7.46 (bd, J=8.0 Hz, 1H) (ArH). Rf 0.63; $[\alpha]_D^{25}$ + 275° (C = 0.30, $CHCl_3$). UV: 278, 291, 305 nm. Colour reaction with $FeCl_3$ reagent gave a greenish-black colour in hot air.

The data is identical with those for cocsuline [13,14].

Cocsuline N-oxide

^{1}H-NMR: 240 dd, 2.41 (N-CH_3), 2.45 m, 2.70 dd, 2.93 m, 3.13 m, 3.30 m, 3.36 (N-CH_3), 3.51 m, 3.72 brd, 3.76 m, 3.81 (O-CH3), 4.13 m, 4.29 (dd, 4.8, 12.3 Hz). 4.44 dd, 6.18 (s, 1H), 6.30 (s, 1H) 6.45 (s, 1H), 6.55 (dd, J = 2.7 Hz 2H), 6.85 (s, 1H), 6.93 (dd, J = 8.0, 2.7 Hz, 2H), 7.08 (bm, 1H), 7.46 (bd, J=8.0 Hz, 1H) (ArH). MS (380°C, 70eV): m/z 578 (M^+, 95), 562 (M^+-16, 45). R_f 0.35; m.p. 210° - 212°C. UV: 278, 291, 305 nm. ν_{max}. (KBr) cm^{-1} 3400, 2910, 1610, 1500, 1220, 1110, 1020, 830. $[\alpha]_D^{25}$ + 264 (C = 0.30, $CHCl_3$).

Colour reaction with $FeCl_3$ reagent gave a greenish black colour in hot air and with a mixture of conc. H_2SO_4 + HNO_3 (1:1) a blue colour indicative of an alkaloid with a dibenzo-1, 4-dioxin moiety [19,20]. In colour ring test, a reddish spot surrounded by a violet ring. These colour reactions were similar to that of cocsuline.

Reduction of cocsuline N-oxide

0.5 mg of the cocsuline *N*-oxide was added to 10% H_2SO_3 (10 cm^3) and set aside for 12 hours. The solution was diluted with H_2O and basified with NH_4OH and extracted with $CHCl_3$ (3 x 20 cm3). The extracts were combined, dried and solvent removed to afford a white powder m.p. 181° - 183°C; R_f 0.63 (EtOAc-Me_2CHOH-25% NH_4OH (9:7:2); UV: 278, 291, 305 nm. These data and TLC properties of the reduction product were identical with that of cocsuline.

Preparation of cocsuline N-oxide

2 mg of cocsuline was dissolved in 5 cm^3 of MeOH and stirred with 15% H_2O_2 and left for 45 min at 25°C. The solution was diluted with a small amount of H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract evaporated to afford a solid. The product was found to be a mixture of two compounds on TLC examination. The less polar of these had identical TLC properties and colour reactions as cocsuline *N*-oxide isolated from *Epinetrum villosum*.

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