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ENICOSTEMA LITTORALE: A NEW SOURCE OF SWERTIAMARIN

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ABSTRACT

Enicostema littorale, a useful medicinal herb was subjected to phytochemical as well as pharmacological investigations. A secoiridoid glycoside, swertiamarin, was isolated and identified on the basis of UV, IR, Mass and NMR spectroscopic measurements. However, pharmacological studies on the blood pressure of rats and ileum of Guinea-pig could not provide significant results.

Introduction

Enicostema littorale Blume is a medicinal herb used in Bokloric system of medicine as bitter tonic, febrifuge, anthelmentic, carminative, mild laxative and antimalarial agent. Sometime in past it was also used as a substitute of cinchona bark and *Swenia chirata* (Bamber, 1916). The plant *E. littorale* belongs to the family Gentianaceae. It is also known as *Enicostema hyssopifolium.*

Studies performed by Dymock et al (1893) on *E. littorale* revealed that the bitter principles from this plant are glycosidat in nature. Practice of various active can-pounds of alkaloids, sterols, reducing sugars and glycosides has been reported by Prasad and Bhusan (Natarajan and Prasad, 1972). Natarajan and Prasad (1972) isolated chloroform soluble and water soluble alkaloids, sterols and volatile oil from this plant. Erythrocentaurin was isolated from *E. littorale* by Ghosal et al. as a major compound in 1974.

Various pharmacological tests have been also reported for *E. littorale* such as effects of the chloroform and water soluble alkaloids on the heart of frog and blood pressure of dog. It was also reported that the water soluble alkaloids have no significant effect on the heart of frog or on blood pressure of dog whereas total chloroform alkaloids produced marked depression of the heart of frog. Significant antibacterial activity of the alcoholic extract was reported against Shigella *dysenteriae* (Ship). However, a little effect was observed on the species of *Shigella dysenteriae*

(Flexner) and *Micr*o*coccus aureus*. Due to this important medicinal uses, this plant was selected for the present chemical and pharmacological investigations which resulted in the isolation, structure elucidation of a pure compound namely Swertiamarin. Its pharmacological activities has also been unreported from this source.

Experimental Part

Extraction Method:

Dried whole plant material, *Enicostetna littorale* was collected near Karachi University Campus in September 1989. These were chopped into small pieces and kept the material with methanol for 15 days at room temperature and this process was repeated at least three time. The extract was concentrated in a vacuum, water was added and the insoluble material was removed by filtration through celite. The filtrate was defatted by extraction with petroleum ether and the soluble part was discarded. The aqueous phase was concentrated and lyophilized to give crude glycosides (24 g).

Isolation of pure compound:

A portion of the crude extract (20 g) was chromatographed over silica gel (500 g) glass column using $CHCl_3$-MeOH-n-PropOH-H_2O (5:6:1:4) as eluent. Two major fractions were collected viz Fr.1 (9 g) and Fr. 2 (5 g). Thereafter, Fr. 1 was chromatographed on silica gel column and semi-preparative RP-HPLC (RP-18 column MeOH-H2O, 35:65, flow rate 8 m1/min.) and a pure compound was obtained as white amorphous powder. This compound was subjected to spectroscopy (UV, IR, Mass, NMR) and was identified as Swertiamarin on the basis of spectroscopic examination (Ghosal et a1., 1974; Nataranjan & Prasad, 1972).

Pharmacological Investigations

In Vivo Experiments

Wistar rats of either sex (200-250 g) *were* anaesthetized with pentothal sodium (40 mg/Kg, i.p). The trachea was exposed and cannulated to facilitate spontaneous respiration. The systemic blood pressure was recorded from the carotid artery via the arterial cannula connected to a pressure transducer (model Statham Pas AC strain gauge transducer) and heart rate was recorded using tachograph. These *were* displayed on Grass model 7D polygraph. Drugs and extracts were slowly injected via the cannula inserted into external jugular vein. The maximum volume of injection was 0.2 ml. The animal was allowed to equilibrate for at least 30 min. before administration of any drug. Mean blood pressure (BP) was calculated by the following formula:

$$\text{MBP} = \text{Diastolic BP} + \frac{\text{Systolic RP - Diastolic BP}}{3}$$

Standard deviation was calculated from the values obtained in each group. Variation in BP were

expressed as % of control values obtained immediately before the ad-ministration of test substance.

In Vitro Experiments

A. Guinea-pig atria:

Guinea-pigs of either sex (400-600 g) were killed by cervical dislocation. Paired atria were removed carefully and mounted into a 10ml tissue bath filed with Krebs-Henselet solution maintained at 32°C and aerated with oxygen. The composition of physiological salt solution was (g/l): NaCl, 6.92; KCI, 0.35; $MgSO_4$, 0.14; KH_2PO_4, 0.16; Glucose, 1.10; $NaHCO_3$, 2.10; and $CaCl_2$, 0.28. The spontaneous atrial contractions were recorded under resting tension of 2g via force displacement transducer (FT-03) on a Grass model 7D polygraph. The preparation was allowed to equilibrate for atleast 30 minutes before administration of any drug.

B. Guinea-pig Ileum

Segments of about 2 cm from guinea-pigs were suspended in a 10 ml tissue bath containing oxygenated Tyrode's solution at 37°C. The composition of Tyrode's solution was (g/l): NaCl, 8.00; KCI, 0.20; $MgCl_2$, 0.10; NaH_2PO_4, 0.05; $CaCl_2$, 0.40; $NaHCO_3$, 2.00 and Glucose, 2.00. Tissues were allowed to equilibrate for 30 min. under a resting tension of 1g and isotonic contraction were recorded on a Bioscience MD_4 recorder.

Results and Discussion

The whole plant including roots was extracted with methanol. The methanolic ex-tract was separated over silica gel for the isolation of glycosidic constituents. A chemical literature search on the constituents of *E. littorale* revealed that no chemical investigation was carried out for iridoid glycosides. But the presence of alkaloids, sterols, volatile oil was reported in literature (Natrangan and Prasad, 1972; Ghosal et al., 1974). During the course of this work we have isolated a secoiridoid glycoside, swertiamarin. Its structure was elucidated on the bases of UV, IR, Mass and NMR spectroscopy. Swertiamarin is a known compound of Swertia japonica and others (Carnelis and Chapelle, 1976; Popove et al., 1987; Plat et al., 1963). The FAB-MS spectra showed a molecular ion peak at m/z 375 [M+H] which corresponded to the molecular formula $C_{16}H_{22}O_{10}$. IR (KBr) spectrum indicated the presence of hydroxyl, carboxylic group and double bond. The presence of unsaturation in the molecule was also noticed in the spectrum of UV. The ^{13}C-NMR spectrum of this compound displayed 16 signal. Out of 16 signals six signals were assigned to glucose moiety while rest of them were alloted to the aglycon portion, on the basis of similar values reported for sweroside (Beek et al., 1982). The chemical structure difference between sweroside and swertiamarin is of hydroxy group, which is present at C-5_{13} in swertiamarin, otherwise both are similar. It is interesting to note that till todate no ^{13}CNMR data of swertiamarin was reported except that of swertiamarin tetracetate (Carnelis and Chapelle, 1976). The ^{1}H-NMR spectrum was used for the determination of number of protons, coupling constants, types and positions of groups along with their stereochemistry. A signal present at 1.74 ppm (ddd, J 6α-6β =

13.5, J 6α-7α = 4.1, J 6α -7β = 12.0 Hz) was assigned to the proton of C-6 exist at α-position while proton present at β-position showed its pressure at 1.84 ppm (ddd J 6β-6α - 13.5 Hz, 16α -7α = 1.7, J 6β-7α = 2.2 Hz). The signal at 2.93 ppm (dd J = 1.8, 9.4 Hz) was assigned to H-9. The protons of 14-7 indicated their presence at 3.90 (dd J 7β -6α = 12.5, 17β-6α = 2.2 Hz, J 7β-7α = 11.4 Hz, H-7α) and 4.35 (ddd, J 7α -6α = 4.2, J 7α -6β = 1.7, J 7α -6β = 11.4 Hz, H-7α) ppm. The signals present at 5.72 (d, J = 1.7 Hz) and 7.62 (d, J = 2.4 Hz) ppm were assigned to H-1 and H-3.

The ^{1}H-NMR of swertiamarin and sweroside are identical in all respect except that sweroside showed an additional signal at 3.90 ppm. For the confirmation of structure and configuration a derivative of swertiamarin (swertiamarin tetra-acetate) was made.

In anaesthetized rats, on pharmacological investigations swertiamarin showed non-significant effect on blood pressure (systolic as well as diastolic) up to 1500 µg/Kg. In vitro studies, swertiamarin upto 100 µg/ml caused no change in force and rate of atrial contraction as shown in fig. B. In guinea pig ileum swertiamarin exhibited very small contraction of smooth muscle at the concentration 100 µg/ml as shown in fig. C.

All the results (*Vivo-Vitro*) showed that the compound was inactive (upto 1500 µg/Kg in vivo and 100 µg/ml in vitro). It might be possible that the compound will give activity on higher doses.

Swertiamarin:

Molecular Weight = 374

Molecular Formula = $C_{16}H_{22}O_{10}$

CH_2OH

UV λ_{max} = 204, 227 nm

IR (KBr) = 3400 (OH), 2910 (C-H), 1705 (C=O), 1620 (C=C), 1060, (C-O-C) cm^1

FAB-MS m/z = 787 (2M+K), 749 (2M+H), 413 (M+K), 397 (M+Na), 375 (M+H), 307, 289, 273, 213, 195, 177, 165, 155.

^{1}H-NMR (300 Mhz, CD_3OD) ppm:
1.74 (ddd, $J_{6\alpha\text{-}6\beta}$ = 13.5 Hz, $J_{6\alpha\text{-}7\alpha}$ = 4.1 Hz, $J_{6\alpha\text{-}7\beta}$ = 12.0 Hz, H-6α), 1.84 (ddd, $J_{6\beta\text{-}6\alpha}$ = 13.5 Hz, $J_{6\beta\text{-}7\alpha}$ = 1.7 Hz, $J_{6\beta\text{-}7\beta}$ = 2.2 Hz, H-6β), 2.93 (dd , J = 1.8, 9.4 Hz, H-9), 3.90 (dd, $J_{7\beta\text{-}6\alpha}$ = 12.5, $J_{7\beta\text{-}6\beta}$ = 2.2 Hz, $J_{7\beta\text{-}7\alpha}$ = 11.4 Hz, H-7β), 4.35 (ddd, $J_{7\alpha\text{-}6\alpha}$ = 4.2 Hz, $J_{7\alpha\text{-}6\beta}$ = 1.7 Hz, $J_{7\alpha\text{-}7\beta}$ = 11.4 Hz, H-7α), 5.72, (d, J = 1.7 Hz H-1), 7.62 (d, J = 2.4 Hz, H-3), Glucose = 4.64 (d, J = 7.8 Hz, H-1'), 3.16-3.50 (m, H-2', 3', 4', 5', 6'B), 3.70 (dd, J = 12.0, 2.1 Hz, H-6'A).

^{13}C-NMR (75.45 MHz, CD_3OD) ppm:

C-1	98.29	C-6	32.01	C-10	121.26	C-3'	77.14
C-13	151.57	C-7	62.99	C-11	169-32	C-4'	70.06
C-4	109.13	C-8	131.49	C-1'	98.92	C-5'	77.86
C-5	62.69	C-9	50.21	C-2'	75.14	C-6'	60.66

Swertiamaria Acetate:

Molecular Weight = 542

Molecular Formula = $C_{24}H_{30}O_{14}$

UV λ McOH max = 236 nm

IR (KBr) = 2980 (C-H), 1750 1710 (C=O), 1620 (C=C), 1050 (C-OC) cm^{-1}

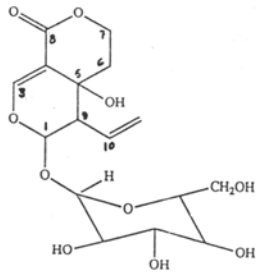
EI-MS m/z = 543 (M+H), 1.2%), 483 (1%), 440 (0.3%), 398 (1.2%), 380 (0.4%), 368 (0.2%), 380, 347 (2.2%), 305, 289 (1.2%), 275 (2.1%), 259 (2.1%), 245 (1.2%), 2.3 (1.2%), 1.65 (1.2%), 161 (2.1%), 155 (0.5%).

^{1}H-NMR (300 MHz, $CDCl_3$) ppm:
1.76 (m, H-6), 2.09-2.01 (s, 4 x Ac), 2.94 (m. H-9), 4.27 (dd, H- 7), 5.37-

4.98 (m, H-8, H-10), 5.47 (d, H-1), 7.48 (s, H-3), Glucose = 4.84 (d, H-1'), 5.37 -4.98 (m, H-2', 3', 4'), 3.75 (m, H-5), 4.13 (dd, H-6).

^{13}C-NMR (75-47 MHz, $CDCl_3$) ppm:

C-1	97.63	C-7	64.60	C-1'	96.85	AC =	169.29
C-3	150.59	C-8	131.38	C-2'	70.58		169.86
C-4	109.69	C-9	50.64	C-3'	71.72		170.47
				C-4'	67.96		170.81
C-5	62.93	C-10	121.31	C-5'	72.33		
C-6	32.45	C-11	164.85	C-6'	61.45		



Swertiamarin

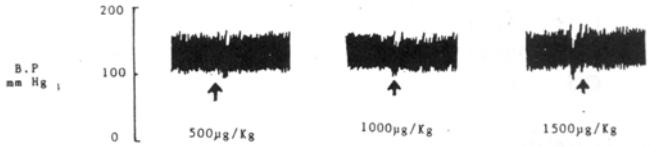


Fig. A: Anaesthetized Rats

Fig. B: Guinea-Pig Atria

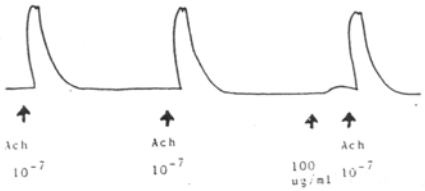


Fig. C: Guinea-Pig Ileum

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