CHEMICAL CONSTITUENTS OF LYCEUM EUROPAEUM

Part I.—Isolation of Lyceamin and β-Sitosterol

M. MANZOOR-I-KHUDA* and (MISS) SITWAT SULTANA

Drugs, Pharmaceuticals and Pesticides Research Division, Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi

(Received January 5, 1968)

A new water-soluble base now designated as lyceamin, m.p. 280–81°C, $C_{19}H_{42}N_4O_7.4H_2O$, and β-sitosterol, m.p. 136–37°C, have been isolated from the aerial part of *Lyceum europaeum*.

The plant *Lyceum europaeum* (synonym *Lyceum barbarum*)[1] grows wild in and around Karachi. The young leaves have been reported to contain hydrocyanic acid[2] and are poisonous to cattle. No systematic chemical investigation on this plant has been reported in the literature so far. Some authors have reported *L. barbarum*, to be different from *L. europaeum*, which has been reported to be an aphrodisiac.[3]

The fresh undried aerial part of the plant, on extraction with ethanol, gave a reddish green extractive, on concentration under reduced pressure. The mainly aqueous concentrate was shaken with ethyl acetate and the aqueous layer (A) was filtered through a pad of cotton. The red-coloured solution, giving a positive test for alkaloid with Dragendorff's reagent, was evaporated to dryness under reduced pressure. The methanol-soluble portion of this residue was adsorbed on neutral alumina and chromatographed on the same material. The methanol-insoluble portion, on crystallisation from water, gave crystals of sodium chloride. The methanol-soluble portion on successive elution with chloroform and chloroform–methanol mixtures from the column gave some crystalline material in the 20% methanol in chloroform elute which gave a positive test with Dragendorff's reagent. This on crystallisation from a small quantity of dry methanol diluted with dry acetone gave shiny rhombic needles of lyceamin, m.p. 280–81°C (in sealed tube), which were highly hygroscopic and readily liquified on short exposures to atmospheric humidity.

The ethyl acetate extract on column chromatography on neutral alumina with successive elution with petroleum ether (40–60°) and benzene gave a crystalline residue from the benzene elute, which on crystallisation from methanol gave a crystalline solid, m.p. 130–31°. It gave a positive Liebermann–Burchard test (violet colour). On further purification β-sitosterol, m.p. and mixed m.p. with an authentic sample m.p. 136–7°C, was obtained. The isolated β-sitosterol gave superimposable IR spectra with that of an authentic sample.

The IR spectra (KBr disc) of lyceamin gave peaks at 3500, 3400, 3065, 3030, 1660i, 1620, 1590i, 1495, 1475, 1458, 1442, 1418, 1405, 1395, 1335, 1243, 1151, 1130, 987, 951, 932, 890 cm^{-1}. It also gave a weak absorption peak in the UV spectra λ_{max} 231 (E^{1}_{1}, 3.6 in ethanol). It had $[\alpha]_D^{31} +1.5 \pm 1.5°$ (*c* 0.79 in ethanol). Lyceamin gave a picrate, m.p. 180–81°C. On hydrolysis with hydrobromic acid a crystalline product having m.p. 223–4°C and ν_{max} 1750, 1710, 1650 cm^{-1} was obtained.

The NMR spectra of lyceamin gave peaks at 6.85, 6.27, 6.22 and 5.36 τ (in D_2O solution with TMS as external standard; intensities equivalent to 17:3:22 protons approximately; 6.27 and 6.22 τ protons integrated for 3 together).

It had a R_f value of 0.366 on elution with butanol, acetic acid and water (4:1:1) on paper No. 2045 Selecta Filter paper (equivalent to Whatman No. 1) for 25 hr. Lyceamin analysed for $C_{19}H_{42}N_4O_7.4H_2O$. A large number of molecules of water of crystallisation was indicated by its mass spectral mol wt (436). The spectral data indicates it to be a hydroindole derivative. The colour reactions for indole nucleus with concentrated nitric acid and *p*-dimethyl aminobenzaldehyde on lyceamin were inconclusive.

Experimental

Unless otherwise stated IR spectra were determined in KBr disc on a Perkin Elmer Model 237E IR Spectrophotometer. Optical rotation was determined in ethanol solution on a Schmidt-Haensch polarimeter. NMR spectra were recorded by Dr. S.H. Zaidi of these Laboratories on a Varian A-60 NMR spectrophotometer using tetramethyl silane as external standard using

*Now at Technological Research Board, Jute Research Institute, Tejgaon, Dacca.

D_2O as solvent. All m. ps are uncorrected. Mass spectrum was taken with a MS-9 mass spectrophotometer by probe injection of sample.

Extraction of Aerial Part of Lyceum europaeum.—The fresh undried aerial part of *Lyceum europaeum* (1.6 kg; 66% moisture) was chopped into small pieces and extracted thrice with ethanol (6.0 l. × 3) at room temperature for about 2 days each time. The solvent was evaporated under reduced pressure and the dark-coloured, mainly aqueous concentrate was shaken with ethyl acetate a number of times. Traces of some dark insoluble material were removed by filtering the solutions through a pad of cotton. The ethyl acetate layer was dried (Na_2SO_4) and evaporated to give a semi-solid mass (18.6 g).

The red-coloured aqueous layer (A) gave a positive Dragendorff's test.

Isolation of β-sitosterol.—The solid from ethyl acetate extract (5.8 g) was dissolved in methanol and evaporated to dryness with some neutral alumina (6 g chromatographic grade). Traces of solvent were removed from the adsorbed alumina by prolonged vacuum treatment in a desiccator. The powdery mass was transferred on to a column of neutral alumina (160 g) made in petroleum ether (40–60°). After elution with petroleum ether (160 ml), the benzene elute collected (5.6 l) gave on evaporation a semi-crystalline material (2.3 g). On crystallisation from methanol, shiny plates were obtained m.p. 130–31°C. It gave a positive (violet) Liebermann-Burchard test. This on further purification, gave β-sitosterol m.p. and mixed m.p. with authentic specimen 136–7°C. It also gave a superimposable IR spectrum with that of authentic β-sitosterol.

Isolation of Lyceamin.—The aqueous layer (A) was evaporated to dryness under reduced pressure. The residue was triturated with dry methanol and the insoluble portion was crystallised from water. The colourless rhombs were identified as sodium chloride through its emission spectra and other tests.

The methanol-soluble portion was evaporated to dryness. A portion of it (1.25 g) was dissolved in methanol and evaporated to dryness with some neutral grade chromatographic alumina (4.0 g). Traces of solvent were removed under reduced pressure in a desiccator and the powdery mass transferred on to a column of neutral alumina (30 g) prepared in chloroform. Fractions (25 ml) were collected and elution continued with methanol–chloroform mixtures till only traces of material came out of the column. The 20% methanol in chloroform fractions were combined together (250 ml) and evaporated under reduced pressure (0.125 g). The crystalline residue gave a positive test with Dragendorff's reagent and was dissolved in a small volume of dry methanol and diluted with dry acetone. Shiny flat needles were deposited from the solution which on repeated crystallisations from the same solvents finally give lyceamin, m.p. 280–81°C, α_D^{31} + 1.5 ± 1.5° (0.79% ethanol) (Found: C, 44.68; H, 9.83; N, 10.94; O, 35.30%, mol wt (mass), 436. $C_{19}H_{42}N_4O_7.4H_2O$ requires C, 44.70; H, 9.80; N, 10.98; O, 34.50%; mol wt, 436). The crystals were highly hygroscopic and absorbed atmospheric moisture spontaneously. It had ν_{max} 3500, 3400, 3065, 3030, 1660i, 1620, 1590, 1495, 1475, 1458, 1442, 1418, 1405, 1395, 1335, 1243, 1155, 1130, 987, 951, 932, 890 $cm.^{-1}$.

Lyceamin Picrate.—Lyceamin (24 mg) was dissolved in dry ethanol (0.5 ml) and a saturated solution of picric acid in ethanol (0.5 ml) added to it. The precipitated material was collected and recrystallised from absolute ethanol to give

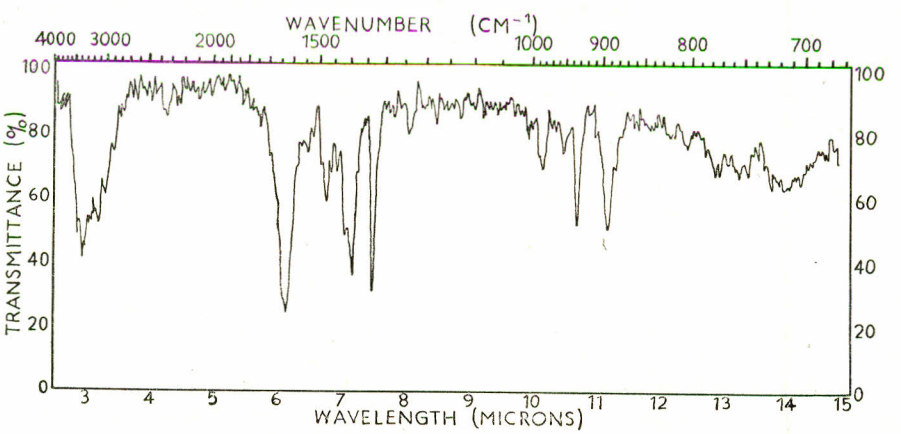

Fig. 1. Lyceamin.

lyceamin picrate, m.p. 180–81°C. It had ν_{max} 1750, 1645, 1570, 1490, 1450, 1425, 1385, 1350, 1275, 1185, 800, 750 cm^{-1}. (only major peaks mentioned).

Hydrolysis of Lyceamin.—Lyceamin (53 mg) was refluxed under N_2 atmosphere with hydrobromic acid (40%; 4 ml) for 2 hr. The reaction mixture was evaporated to dryness and the crude hydrobromide crystallised from ethanol and acetone mixture to give a hydrobromide, m.p. 218–221°C. This was not as hygroscopic as lyceamin. It had ν_{max} 3000, 2850, 1750, 1710, 1650, 1200, 1000, 950, 930, 885 and 835 cm^{-1} (KBr pellet).

Acknowledgement.—The authors wish to thank Dr. M. Barbier of Institute of Chemistry for Natural Products, Gif-Sur-Yvette, France and Dr. M.A. Salaque of these Laboratories for their kind help with the elemental and mass spectral mol wt analysis.

References

1. S.M.H. Jafri, *The Flora of Karachi* (The Book Corporation, Karachi) p. 299.
2. R.N. Chopra, I.C. Chopra, K.L. Handa and L.D. Kapur, *Indigeneous Drugs of India* (U.N. Dhur Sons,Calcutta), p. 546.
3. K.M. Nadkarni, *Indian Materia Medica* (Popular Book Depot, Bombay), p. 756.