CHEMICAL STUDIES ON BUXUS PAPILOSA—ISOLATION OF TWO NEW ALKALOIDS BUXPAPINE AND BUXPAPAMINE

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Two new alkaloids provisionally named *buxpapine*, $C_{27}H_{34}N_2O$ and *buxpapamine*, $C_{28}H_{30}N_2O$ have been isolated from strong base fraction of *B. papilosa*.

Buxus papilosa is a shrub which often grows gregariously on limestone. In Pakistan, it is available[1] in Hazara, Rawalpindi, Kala Chitta hills and Sakesar. Extracts of *Buxus papilosa*, like those of *Buxus sempervirens* L., have been used as febrifuge, for rheumatism and for many other ailments. Many *Buxus* species such as *sempervirens*,[2] *microphylla*,[3] *balearica*[4] and *malayana*[5] have been found to contain a number of alkaloids. However, no work seems to have been done on *B. papilosa*. We have now isolated two alkaloids in pure state from the strong base fraction of the crude alkaloidal mixture. In addition, two other alkaloids have been obtained from the same fraction and further work is in progress on their purification.

Buxpapine, $C_{27}H_{34}N_2O$, showed IR bands at 1647 and 888 cm^{-1} which may be ascribed to a terminal methylene group; NMR showed peaks for two N-methyl groups at 7.62 and 7.82 τ.

Buxpapamine, $C_{28}H_{30}N_2O$, showed IR bands at 3050 and 1655 cm^{-1} which may be ascribed to the presence of a double bond in the molecule and NMR peaks for three N-methyl groups at 7.80, 8.00 and 8.2 τ.

Experimental

Dried leaves and stems of *Buxus papilosa* (5.0 kg), ground to a fine powder were percolated with 95% ethyl alcohol (1.5 gallons). Five such extractions were sufficient for exhausting the plant of their alkaloidal content. The combined extracts were concentrated under reduced pressure in a cyclone evaporator. The semi-solid residue thus obtained was treated with 2N acetic acid and the resulting suspension kept overnight, when most of the chlorophyll and fatty part settled down. The filtered solution was basified with ammonium hydroxide and the alkaloids extracted with ethyl acetate. The ethyl acetate extract, after charcoaling and drying ($CaSO_4$), was filtered and the solvent removed under reduced pressure. The crude alkaloidal residue (55.0 g) was again dissolved in 2N acetic acid (400 ml) and extracted with chloroform (3×150 ml). The chloroform extract was washed with 2N ammonium hydroxide, then with water, dried ($CaSO_4$), filtered and the solvent removed. The residue was dissolved in 2N HCl (50 ml) and chloroform (50 ml). After agitation, the chloroform layer was separated and again shaken with 2N HCl (50 ml). The combined acidic solutions were basified and extracted with chloroform to give weak bases (2.08 g). The original acetic acid solution of the crude alkaloid, now freed of weakly basic fraction, was basified to pH 7.0 with ammonium hydroxide and extracted with chloroform to get moderately strong bases (4.5 g). The remaining solution basified with strong ammonium hydroxide to pH 9.5 and extracted with chloroform to give strong bases (17.0 g). The aqueous layer then showed a negative Draggendorff test for alkaloids.

Chromatography of Strong Base Fraction.

The strong base fraction (17.0 g) was dissolved in a minimum amount of chloroform and chromato graphed on a column of alumina, 200 g (May & Baker), prepared in benzene. The column was eluted with dry benzene, a mixture of benzene and chloroform, chloroform and finally with ethyl alcohol. The following fractions of 400 ml each were collected.

Fractions I–IV with benzene; V and VI with benzene: chloroform (4:1); VII with benzene: chloroform (3:2), VIII with pure chloroform; IX with ethyl alcohol.

Fraction I (Provisionally Named as Buxpapine).— The solvent was removed from the fraction under reduced pressure. The residue was crystallized from acetone to give buxpapine 1.14 g, m.p. 111–112°C, $[\alpha]_D$ +7.5 (*c* 4.8, $CHCl_3$). (Found: C, 80.11; H, 8.59; N, 7.03% $C_{27}H_{34}N_2O$ requires C, 80.60; H, 8.45; N, 6.96%).

The IR spectrum (KBr) showed bands at 2915, 2874, 2770, 1647, 1449, 1377, 1014, 888, 791 and 747 cm^{-1}. The NMR spectrum ($CDCl_3$, TMS internal standard) showed peaks at 4.7 (singlet, broad), 7.5 (singlet, broad), 7.6 and 7.8 (2 *N*-methyls), complex peaks between 8.0–8.7 region, 9.1 (a sharp singlet, probably a tertiary methyl), 9.2 (a doublet, probably a secondary methyl) and 9.3 τ (a doublet, probably a secondary methyl)

Fraction II, III and IV (Provisionally Named as Buxpapamine).—These fractions, after evaporation of the solvent, gave residues (260 mg) which were found to be identical by thin layer chromatography. On repeated crystallization from acetone, it melted at 205–206°C, $[\alpha]+93°$ (*c* 1.28, $CHCl_3$). (Found: C, 82.73; H, 7.35; N, 6.86%. $C_{28}H_{30}N_2O$ requires C,81.95; H, 7.31; N, 6.83%).

The IR spectrum (KBr) showed bands at 3050, 2950, 2860, 2780, 1655, 1455, 1370, 885 cm^{-1}. The NMR spectrum ($CDCl_3$, TMS internal standard) showed peaks at 7.8, 8.0, 8.18 (three sharp peaks; probably 3 *N*-methyl), complex peaks between 8.3–8.8, 9.05, 9.12 (two sharp peaks; 2-tertiary *C*-methyl), 9.28 (a doublet, a secondary *C*-methyl) and two peaks at 9.68 and 9.6, which are centred at 9.65 τ, probably a doublet (J=4 c/s) due to cyclopropyl methylene.

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