

## PLANT ALKALOIDS. PART I. *BERBERIS FLORIBUNDA*, WALL. EX DON.

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The root of *Berberis floribunda*, Wall. ex Don. has yielded eight alkaloids, e.g., oxyacanthine, berbamine, berberine, *epiberberine*, palmatine, dehydrocorydalyne, jatrorrhizine and columbamine. *B. floribunda* appears to be the first instance of bearing *epiberberine*, which was prepared by Perkin during his synthetical studies on berberine and cryptopine alkaloids (*J. Chem. Soc.*, 1918, 113, 492).

*Berberis floribunda*, Wall. ex Don. (Family *Berberidaceae*) is an erect shrub, 10 ft. high. It grows in Nepal, from where the plant material was procured and identified by Dr. S. K. Mukerjee, Curator of the Herbarium, Indian Botanic Garden, Sibpur, Calcutta. Several Indian species of *Berberis* have been examined chemically (Henry, "The Plant Alkaloids," 1949, p. 328), but they have not yielded so many alkaloids, and it is interesting to obtain as one of the constituents, *epiberberine*, for the base has so far been of synthetical interest (Perkin, *J. Chem. Soc.*, 1918, 113, 492), though Goto and Kitasato (*ibid.*, 1940, 1234) isolated *l*-tetrahydro-*epiberberine* or *sinactine* from the roots of *Sinomenium acutum*, Rehd. and Wils. (Family *Menispermaceae*). The following flow sheet indicates the method of isolation of the alkaloids from the plant.

### EXPERIMENTAL

Air-dried, finely powdered (60 mesh) root (1 kg.) of *B. floribunda* was extracted with ethanol (95%). The solvent was removed under reduced pressure and the sticky brown residue was treated with iced water (500 c.c.) and filtered to remove resinous matter. The clear dark brown solution was cooled to 0° and made alkaline with ammonia (25%), when a sticky amorphous solid separated and the mixture was extracted with ether: A. The ether layer. B. The aqueous layer.

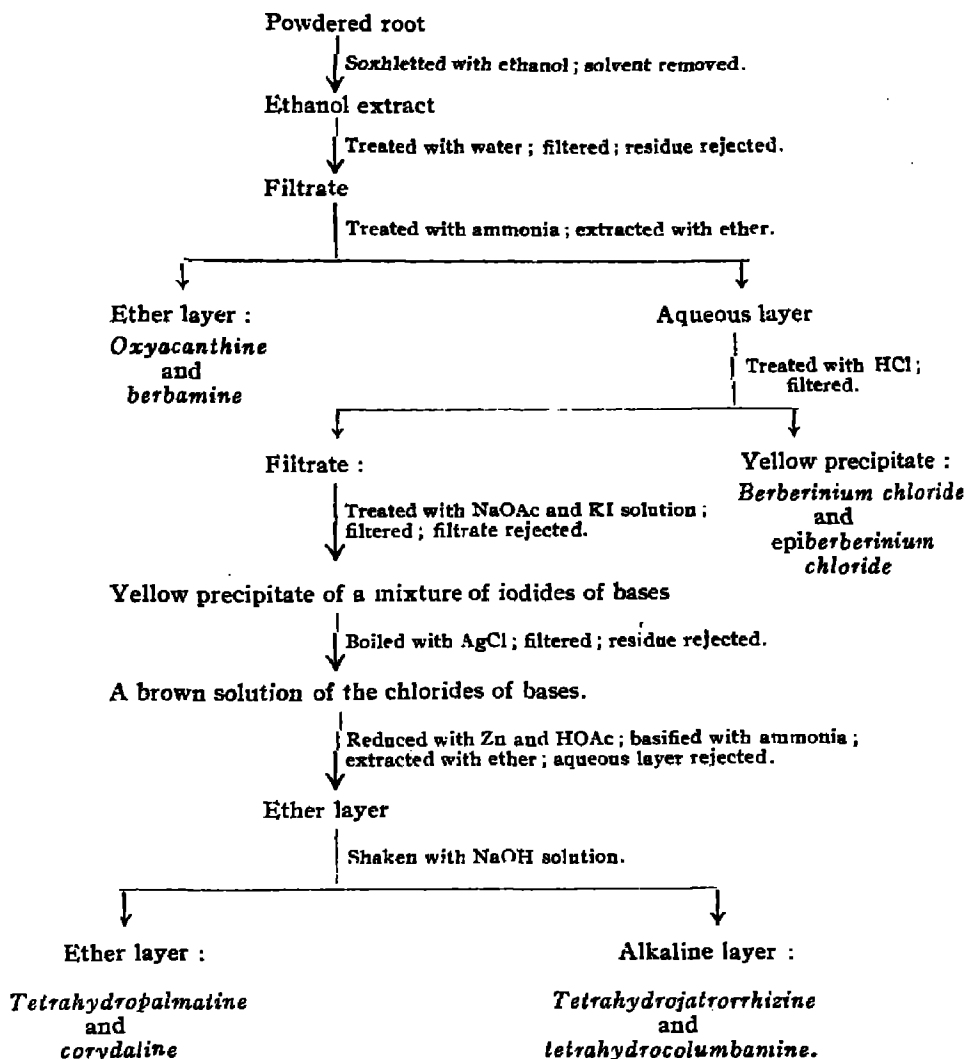
#### A. Treatment of the Ether Layer

On removal of ether, a sticky brown residue was left behind. This was soxhletted with dry ether when most of the residue dissolved. The mixture was filtered and the filtrate was concentrated and kept for three days in an ice box when a small quantity of practically colorless crystals separated, which were filtered and then dissolved in hydrochloric acid (5%) and kept in the ice box for several days, when a very small quantity of the crystals of a hydrochloride appeared which were filtered: I. The crystals. II. The filtrate.

I. *Identification of Oxyacanthine from the Crystals.*—The crystals were dissolved in warm water and basified with ammonia, which precipitated an amorphous oxyacanthine base. The base was taken up in ether and the ethereal solution on long standing in an ice box yielded a very small quantity of colorless needles of oxyacanthine which on crystallisation from methanol melted at 216° *in vacuo*. (Found: C, 73.1; H,

6.9; N, 4.6.  $C_{27}H_{40}O_4N_2$  requires C, 72.99; H, 6.6; N, 4.6 per cent). The needles did not depress the m.p. of oxyacanthine, m.p.  $216^\circ$  in *vacuo*, isolated from *Mahonia Griffithii*, Takeda<sup>1</sup> (Chatterjee and Guha, *J. Amer. Pharm. Assoc.*, 1950, **39**, 182) and gave all the colour reactions for oxyacanthine as described in the above paper.

*An Outline of the Method of Isolation of the Alkaloids*



II. *Identification of Berbamine from the Filtrate.*—The filtrate was cooled and treated with sodium nitrate solution, when a small quantity of crystals of berbamine nitrate was deposited. The free base was liberated as above with ammonia, which on crystallisation from methanol melted at  $155^\circ$  with decomposition. (Found: C, 72.8; H, 6.9.  $C_{27}H_{40}O_4N$  requires C, 72.99; H, 6.6 per cent). The m.p. and colour

reaction of the base indicate it to be berbamine, which was confirmed by mixing it with an authentic sample of berbamine (*loc. cit.*) and determining the m.p. 156° (decomp.)

#### B. Treatment of the Aqueous Layer

The clear brown aqueous solution was treated with hydrochloric acid to give an 1% solution and allowed to stand overnight in an ice box when a mixture of orange and yellow chlorides separated and filtered: I. The crystals. II. The filtrate.

I. *Examination of the Crystals.*—The mixture of the chlorides were fractionally crystallised from methanol in which orange crystals were less soluble than the yellow ones. Yields of (a) yellow chloride, 5 g. and (b) orange chloride, 0.01 g.

*Identification of the Yellow Chloride as Berberinium Chloride.*—The chloride was dissolved in water, and was basified with sodium carbonate solution, and on cooling yellow silky needles of the free base were obtained. They were purified by repeated crystallisation from ethanol. (Found:  $\text{CH}_2\text{O}_2$ , 10.2.  $\text{C}_{20}\text{H}_{18}\text{O}_5\text{N}$ ,  $5.5\text{H}_2\text{O}$  requires  $\text{CH}_2\text{O}_2$ , 10.17 per cent).

*The Acetone Compound.*—The yellow chloride (0.1 g.) was dissolved in water (10 c.c.) and was mixed with 10% sodium hydroxide solution (4 c.c.), heated to 50°, mixed with acetone (5 c.c.) and allowed to cool, when lemon-yellow crystals gradually separated, m.p. 168° (decomp.). The crystals did not depress the m.p. of an authentic sample of the acetone compound of berberine, m.p. 168° (decomp.).

*The Picrate.*—An ethanolic solution of picric acid was added to a warm ethanolic solution of the chloride, when on cooling glistening yellow needles of the picrate, m.p. 238° (decomp.) were obtained. The crystals did not depress the m.p. of berberine picrate, m.p. 237-39° (decomp.).

*Tetrahydroanhydroberberine.*—The yellow chloride (2 g.) was dissolved in water (75 c.c.) and reduced by zinc dust and acetic acid by warming on the water-bath until the colour of the mixture became pale yellow. The mixture was filtered, cooled and treated with ammonia till the precipitated zinc hydroxide went in solution. It was then extracted repeatedly with ether, and on removal of the ether, a colorless crystalline product was obtained (1.4 g.), which was crystallised from methanol, m.p. 172°. It did not depress the m.p. of an authentic sample of tetrahydroanhydroberberine, m.p. 173°.

*Identification of the Orange Chloride as epiBerberinium Chloride.*—The crude orange crystals were dissolved in water, treated with animal charcoal and then mixed with concentrated hydrochloric acid, when orange crystals began to separate after some time. (Found: C, 64.0; H, 4.8.  $\text{C}_{20}\text{H}_{18}\text{O}_5\text{NCl}$  requires C, 64.6; H, 4.8 per cent).

The acetone compound was prepared by the method of obtaining the acetone compound of berberine, as yellow prisms, m.p. 162°.

The picrate of the base was also obtained by the method of preparation of berberine picrate. It melted not very sharply at 222° (decomp.) as mentioned in literature (Perkin, *loc. cit.*); berberine picrate, however, melts at 239° (decomp., Pictet and Gams, *Ber.*, 1911, 44, 2485).

*Tetrahydroanhydroepiberberine.*—A sample of the chloride was reduced by zinc and acetic acid by the method of reduction of berberinium chloride to obtain colorless

prisms of tetrahydroanhydroepiberberine, m.p.  $170-71^{\circ}$ . (Found: C, 70.5; H, 6.5.  $C_{20}H_{21}O_4N$  requires C, 70.8; H, 6.2 per cent).

II. *Examination of the Filtrate*.—The filtrate (500 c.c.) was treated with sodium acetate solution, followed by an aqueous solution of potassium iodide (1:1) which precipitated the remaining bases as iodide (7.2 g.) which was filtered and the filtrate rejected.

The iodide was then suspended in water (300 c.c.) and was digested with freshly precipitated silver chloride (10 g.) for 15 hours. The hydrochloride of the bases thus formed went in solution and was filtered and was immediately reduced by zinc and acetic acid (40 c.c.) and concentrated hydrochloric acid (20 c.c.) by warming on a water-bath. The reduced solution was filtered, cooled, and treated with ammonia till the precipitated zinc hydroxide redissolved. The solution was then extracted exhaustively with ether and the ether layer was washed with 5% sodium hydroxide solution. (i) The ether layer. (ii) The alkaline solution.

(i) *Treatment of the Ether Layer*.—The ethereal solution was evaporated and the pale yellow residue (2.5 g.) was dissolved in methanol. On fractional crystallisation colorless prisms, m.p.  $135^{\circ}$ , separated first and the mother-liquor on concentration deposited crystals, m.p.  $144^{\circ}$ .

*Identification of the Crystals of M. P.  $144^{\circ}$  as Tetrahydroanhydropalmatine*.—The crystals did not depress the m.p. of an authentic sample of tetrahydroanhydropalmatine, m.p.  $144^{\circ}$ . (Found: C, 70.6; H, 7.2; N, 3.8.  $C_{21}H_{22}O_4N$  requires C, 70.98; H, 7.04; N, 3.94 per cent).

*Identification of Crystals of M. P.  $135^{\circ}$  as Corydaline*.—The crystals formed the hydrochloride of m.p.  $206^{\circ}$  and the nitrate of m.p.  $196-97^{\circ}$ . (Found: C, 71.4; H, 7.7; N, 3.8.  $C_{22}H_{23}O_4N$  requires C, 71.55; H, 7.3; N, 3.79 per cent).

(ii) *Treatment of the Alkaline Solution*.—The solution was cooled in ice and acidified with hydrochloric acid and was basified with ammonia and the crude reduced base which did not keep well in air was dissolved in methanol and was treated with tincture of iodine and warmed on the water-bath for one minute and then filtered. The filtrate was decolorised with a mixture of dilute hydrochloric acid and sodium bisulphite and was allowed to cool. Two crops of crystals were obtained. Both were recrystallised from methanol, m.p.  $210^{\circ}$  and  $223^{\circ}$  respectively. The crystals of m.p.  $210^{\circ}$  did not depress the m.p. of jatrorrhizine iodide, m.p.  $210-12^{\circ}$ . Since columbamine iodide melts at  $223-24^{\circ}$ , it appears that the crystals of m.p.  $223^{\circ}$  are of columbamine iodide. As the quantity of the crystals was very small, it was not possible to examine the base in detail. The percentage of nitrogen present in the crystals agrees with that of nitrogen in columbamine iodide. (Found: N, 3.2.  $C_{20}H_{20}O_4N.I$  requires N, 3.0 per cent).