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Chemical Investigation of *Khet-papra*

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Chemical examination of *Oldenlandia biflora* has shown that the plant does not contain any alkaloidal material, but only ursolic acid; the same compound has also been isolated from *Oldenlandia herbaceae*. Commercial *Khet-papra* (identified as *Fumaria parviflora*) gave protopine as the chief basic constituent. This has been confirmed by the isolation of protopine from fresh *Fumaria parviflora*. 'Biflorine', previously reported to have been isolated from *Oldenlandia biflora*, has been found to be identical with protopine.

OLDENLANDIA biflora (*Rubiaceae*) has been classified by Chopra *et al.*[1] under drugs 'whose investigation is likely to be useful'. Hence it was considered of interest to undertake its systematic chemical investigation. Chauhan and Tewari[2] have reported the isolation of two alkaloids, biflorine, $C_{17}H_{17}O_4N$, m.p. 206°, and biflorone, $C_{17}H_{15}O_4N$, m.p. 98°, from this plant. However, their work has been confined to the preparation of a number of derivatives of the two alkaloids and a study of their interconversion.

An authentic sample of *Oldenlandia biflora* was collected from the suburbs of Madras during 1956. Systematic examination of this plant showed that it did not contain even traces of alkaloidal material. The only crystalline organic substance that could be got from this plant was the triterpene acid, ursolic acid. Treatment of the whole plant with acetone, after extraction with light petroleum, gave the acid which was purified by esterification with diazomethane and chromatography of the crude ester. Crystallization from dilute methanol gave methyl ursolate, m.p. 166°-68°, yielding an acetate, m.p. 241°-43°, their identity being confirmed by comparison with authentic specimens obtained from Dr. D. E. White, University of Western Australia.

Oldenlandia herbaceae, another plant belonging to *Rubiaceae*, was likewise found to contain considerable quantities of ursolic acid. The plant showed the presence of basic material only in traces in alkaloidal assays.

It was suspected that the commercial *khet-papra* that Chauhan and Tewari[2] worked with was probably wrongly identified as *Oldenlandia biflora*. Specimens of commercial *khet-papra* were obtained by us from various sources and were identified as *Fumaria parviflora*, belonging to the family *Fumariaceae*. Extraction of the plant with light petroleum gave considerable quantities of a wax, which is probably ceryl alcohol. Percolation with cold acetone and working up as described in the experimental section gave an alkaloid, m.p. 205° (same as that reported for biflorine[2]). The alkaloid showed the presence of a methylenedioxy group and exhibited no rotation. Methoxyl groups were absent. (Chauhan and Tewari[2] have reported that biflorine has one methoxyl and no 'piperonal' groups; further, its specific rotation has been recorded as −135·4.) These properties, together with the fact that protopine is an ubiquitous alkaloid in the *Fumariaceae* as revealed by the systematic work of Manske[3], made us suspect that the alkaloid isolated by us might be protopine. This was confirmed by analysis of the alkaloid and its picrate, and a mixed melting point determination with authentic protopine. The ultraviolet spectra of the base, its hydrochloride and methiodide also exhibited the characteristic shifts that have been recorded for protopine and ascribed to *trans*-annular interaction[4,5].

We could not, however, isolate an alkaloid corresponding to 'biflorone' from the mother liquors, despite a careful search. Nor could we oxidize protopine to 'biflorone' employing the conditions used for converting biflorine to biflorone[2].

A fresh sample of *Fumaria parviflora*, collected from Ootacamund in June 1957, also yielded protopine, confirming that this alkaloid is the basic constituent of *khet-papra*.

After the completion of this work, samples of biflorine and biflorone were received through the kind courtesy of Prof. J. D. Tewari. Biflorine was found to be identical

with protopine (melting point and mixed melting point) obtained by us.

Experimental procedure

Ursolic acid from Oldenlandia biflora — Powdered plant material (whole plant; 1 kg.) was repeatedly extracted with cold light petroleum. It was then percolated in the cold with acetone and the acetone extract was evaporated. The greenish mass from three such extractions was washed with acetone to give a pale green crystalline powder (2 g.), m.p. 250°-54°.

A suspension of the substance (1 g.) in methanol was treated with diazomethane (from 5 g. of nitrosomethylurea). The ethereal solution of the product, after washing with alkali and drying, was evaporated to yield a gum (1 g.) which was chromatographed in benzene solution through a column of alumina (60 g.), the eluate being collected in 25 ml. fractions. The first fraction gave a small amount of a gum while fractions 2-6 gave a solid on evaporation. Recrystallization from dilute methanol gave methyl ursolate (0·6 g.), m.p. 166°-68°, mixed m.p. with an authentic specimen, 168°-69°, $[\alpha]_D^{30°}$ (c, 1 in chloroform), +70. (Found: C, 79·3, 79·5; H, 10·8, 10·4. $C_{31}H_{50}O_3$ requires C, 79·2; H, 10·6 per cent.) The acetate was obtained by leaving aside methyl ursolate (0·1 g.) with acetic anhydride (1 ml.) for 24 hr., and diluting with water. Crystallization from alcoholic acetic acid gave colourless stout needles, m.p. and mixed m.p. 241°-43°.

Ursolic acid from Oldenlandia herbaceae — Powdered plant material (whole plant; 1 kg.) was first extracted with light petroleum and then with acetone. Evaporation of the acetone extracts and washing the residue with more of the fresh solvent gave crude ursolic acid (2 g.). Esterification with diazomethane followed by chromatography gave methyl ursolate (0·5 g. from 1 g. of the crude acid), m.p. and mixed m.p. 163°-65°.

Extraction of commercial khet-papra — Dry, powdered *khet-papra* (8 kg.) was percolated in the cold with light petroleum for two days. Distillation of the solvent left a greenish residue which was digested with a small quantity of acetone and filtered. The residue was again washed with a small quantity of acetone, which removed the green colour, leaving ceryl alcohol (20 g.), m.p. after two crystallizations from alcohol, 81°-82°. (Found: C, 82·0; H, 13·8. $C_{26}H_{54}O$ requires C, 81·7; H, 14·1 per cent.) Two further extractions with the same solvent gave more (15 g.) of the substance.

The plant material (8 kg.), which had been exhausted with petrol, was percolated in the cold with acetone for 2 days. The solvent was then distilled off and the combined residue from three such extractions digested with water (200 ml.) containing dilute sulphuric acid (10 ml.). The mixture was left aside for 4 hr. and filtered. After removal of non-basic material by ether extraction, the aqueous solution was cooled, basified with ammonia and thoroughly extracted with chloroform. The dried (Na_2SO_4) chloroform extract was distilled, and the residue crystallized from alcohol. The solid was then filtered in chloroform solution through a short column of alumina. Evaporation of the solvent and recrystallization from alcohol gave greyish white crystals of protopine (1·6 g.), m.p. and mixed m.p. 205°. (Found: C, 68·1; H, 5·4; N, 4·0; —OMe, 0; —NMe, 8·3. $C_{20}H_{19}O_5N$ requires C, 68·0; H, 5·4; N, 4·0; —NMe, 8·2 per cent.) The picrate, crystallized from acetic acid, had an indefinite m.p., decomposing at about 240°. (Found: C, 54·1; H, 3·7; N, 9·5. $C_{26}H_{22}O_{12}N_4$ requires C, 53·6; H, 3·8; N, 9·6 per cent.)

Extraction of fresh Fumaria parviflora — Fresh plant collected from Ootacamund in June was dried and powdered; the powder (80 g.) was extracted twice with petrol and then twice with acetone. The combined acetone extracts were evaporated and worked up as before to yield protopine (10 mg.), m.p. and mixed m.p. 205°-6°.

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