

ISOLATION OF LAPACHOL FROM *DIPHYSA ROBINOIDES*

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Previous chemical studies of heartwood and root from genera of Bignoniaceae and Proteaceae have shown the presence of the compound known as lapachol, an important quinone (1) having antimicrobial characteristics (2,3). This work describes the isolation of lapachol from *Diphyssa robinoides* Bent (Leguminosae).

**PLANT MATERIAL.**—The plant material was collected near the central coast of Veracruz State, México, where the tree grows wild on a limited scale, and was authenticated by the Herbarium of the Universidad Veracruzana at Xalapa, where a voucher specimen has been deposited. There is no previous report of the isolation of lapachol from other species of the genus *Diphyssa*.

**EXTRACTION AND ISOLATION OF LAPACHOL.**—Dry wood (4 kg), when sawed up, gave 300 g of a powdered material with toxic effects on human skin. This material was extracted with EtOH at room temperature. The extract was concentrated in vacuo, and the ethanolic concentrate extracted with C<sub>6</sub>H<sub>6</sub>. The C<sub>6</sub>H<sub>6</sub> fraction was evaporated to dryness and extracted with a mixture of hexane-Me<sub>2</sub>CO (8:2) to give a yellow residue (800 mg). Purification by recrystallization from the same solvent gave 500 mg of yellow crystals with mp 126-127°. This product gave a positive test for quinone with alkali solutions.

The mass spectrum gave data corresponding to C<sub>15</sub>H<sub>14</sub>O<sub>3</sub>, molecular weight 242, and the ms, <sup>1</sup>H-nmr and ir spectra were identical with those reported for lapachol (4,5).

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ALKALOIDS FROM EMBRYO OF THE SEED OF *NELUMBO NUCIFERA*

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The embryo of the seed of *Nelumbo nucifera* Gaertn. (Nymphaeaceae) (embryo loti) has been used in traditional medicine as an antifebrile, antipsychotic, and antihypertensive agent (1).

Chao *et al.* (2) reported the isolation of liensinine from the embryo loti of Chinese origin, which had antihypertensive activity. On the other hand, Tomita *et al.* (3) reported the isolation of isoliensinine from the embryo loti of Taiwan origin, which is inactive. Despite the absence of liensinine, we have observed that the aqueous extract of the embryo loti of Taiwan origin shows antihypertensive activity on spontaneously hypertensive rats.

Our interest has been directed to the investigation of the constituents of the embryo loti, with the aim of isolating biologically active substances; this paper describes the isolation of four alkaloids, i.e., isoliensinine, neferine, (±)-armepavine, and 4'-methyl-N-methylcoclaurine.

The antihypertensive principle was characterized as neferine.

The isolation of 4'-methyl-N-methylcoclaurine is the first report of its occurring as a natural product, though it is synthetically known as a product from cleavage of neferine by sodium in liquid  $\text{NH}_3$  (4).

This is the first time that co-occurrence of bis-coclaurine-type alkaloids (isoliensinine and neferine) and coclaurine-type alkaloids [(±)-armepavine and 4'-methyl-N-methylcoclaurine] in the embryo loti is reported.

#### EXPERIMENTAL

**EXTRACTION AND ISOLATION.**—Dried and powdered embryo loti (168 g) was extracted seven times with hot MeOH. The MeOH extract was concentrated in vacuo to a syrup, mixed with 3% tartaric acid solution and filtered. The filtrate was made alkaline with  $\text{NH}_4\text{OH}$  solution and extracted with  $\text{Et}_2\text{O}$ . The  $\text{Et}_2\text{O}$  solution was treated with 3% NaOH solution. The aqueous solution was adjusted to pH 10 with  $\text{NH}_4\text{Cl}$  and then extracted with  $\text{Et}_2\text{O}$ . The  $\text{Et}_2\text{O}$  extract dissolved in  $\text{CHCl}_3$  was treated successively with pH 5.5 and 5.0 McIlvaine buffer solutions. Chromatography of the crude alkaloids from the pH 5.5 McIlvaine buffer solution on a silica gel column eluting with a  $\text{CHCl}_3/\text{MeOH}$  gradient afforded isoliensinine (21 mg) and (±)-armepavine (16 mg). Chromatography of the crude alkaloids from the pH 5.0 McIlvaine buffer solution on a silica gel column eluting with a  $\text{CHCl}_3/\text{MeOH}$  gradient afforded neferine (37 mg) and 4'-methyl-N-methylcoclaurine (13 mg).

All four compounds were identified by comparison of their physical and spectral properties with those reported in the literature (3-5).

**TEST FOR ANTIHYPERTENSIVE ACTIVITY.**—Spontaneously hypertensive rats were anesthetized with pentobarbital Na, 50 mg/kg, ip and urethane, 1.75 g/kg, sc. Each sample (10 mg/kg) was administered intravenously to those rats through the femoral vein and tested for its antihypertensive activity. Blood pressure was directly measured by carotid artery cannuration.

Full details of the isolation, identification, and antihypertensive activity of the compounds are available on request to the senior author.

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#### METABOLITES FROM THE FERMENTATION OF *ULOCLADIUM BOTRYTIS*

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Fungal metabolites have gained attention recently in view of their chemical diversity. There are no reports on the chemical constituents of *Ulocladium botrytis* Preuss, a seed-borne fungus of pearl millet (*Pennisetum typhoides*) and hence the present investigation.

#### EXPERIMENTAL

Monosporic cultures of *U. botrytis* in modified Czapeks medium (1) were incubated at 27-29° for 15 days. The whole cultures were extracted in a liquid-liquid extractor using  $\text{CHCl}_3$ . Silica gel column chromatography of the extract using petroleum ether and  $\text{C}_6\text{H}_6$  yielded dodecane (ir,  $^1\text{H}$  nmr, ms) (2) and 9,10,12,13-tetrahydroxyheneicosanoic acid (ir,  $^1\text{H}$  nmr, ms) (3,4). The latter compound was earlier reported from the fungus *Haematomma ventosum* (4).

The details of isolation and identification of the compounds are available on request.