The Presence of l-Maackiain and Pterocarpin in Callus Tissue of *Sophora angustifolia*

Chemical studies on constituents in callus tissues of medicinal plants are carried out in our laboratory, and the productions of tobacco alkaloids\(^1\) and phytosterols\(^9\) by tobacco calluse tissue were previously reported. Now we wish to report the presence of a large amount of l-maackiain (I) and a small amount of pterocarpin (II) in callus tissue of *Sophora angustifolia* (Leguminosae, Japanese name; ku-rā-ra) which is widely distributed in Japan and whose root is used as stomachic, diuretic, and agricultural anthelmintic.

The callus tissue derived from aseptically germinated seeds of *Sophora angustifolia* was grown on White's agar medium containing 1 mg/liter of 2,4-D (2,4-dichlorophenoxyacetic acid), 0.5 mg/liter of kinetin and 1 g/liter of Difco yeast extract. The callus tissue was subcultured at six weeks intervals for about three years.

The callus tissue (1,210 g wet weight) collected was preserved in methanol for two weeks and homogenized with 300 ml cold methanol in a Waring blender. The callus tissue (45.2 g dry weight) was filtered, and the filtrate was evaporated under reduced pressure. The concentrated aqueous solution was extracted with chloroform and the chloroform solution was shaken with \(2N\) NaOH (3 x 200 ml). The NaOH solution after acidification was again shaken with chloroform. The chloroform solution was concentrated to 50 ml, and column chromatographed using silica gel as adsorbent and benzene/acetic (5:1) as developing solvent.

The fluorescent substance was first separated and the following pale yellow substance (about 700 mg) was eluted out. This second substance was several times recrystallized from aqueous methanol.

The needle crystal (I) obtained (532 mg, yield=1.18% dry weight) showed \(C_{16}H_{12}O_4\cdot\frac{1}{2}H_2O\), mp 178—179\(^\circ\), \([a]_D^254 = -254^\circ\), \(c=0.871\), in MeOH, \([M]+m/e 284.067\) (Calc. 284.068 for \(C_{16}H_{12}O_4\cdot\frac{1}{2}H_2O\)), and no depression with authentic sample of l-maackiain hemihydrate. The UV, IR, and NMR spectra were identical with those of l-maackiain.

The crystal identified with l-maackiain was further methylated with diazomethane. The methylated compound (II) showed mp 154.5—156\(^\circ\) and no depression with authentic sample of l-pterocarpin.

The presence of pterocarpin was also detected in neutral fraction of the callus tissue by thin-layer chromatography and gas-liquid chromatography.

It is noteworthy that l-maackiain yield (1.18%) from plant callus tissue was much higher than visnagin (0.31%) by Staba, et al.\(^{4,4}\) The coexistence of l-maackiain and pterocarpin in plant callus tissue is also of interest from chemotaxonomical point of view.

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