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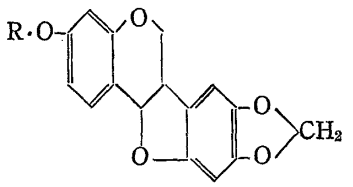
The Presence of *l*-Maackiain and Pterocarpin in Callus Tissue of *Sophora angustifolia*

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[2] 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-ra-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 (3×200 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/acetone (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.



I: R=H II: R=CH_3

The needle crystal (I) obtained (532 mg, yield=1.18% dry weight) showed $C_{16}H_{12}O_5 \cdot \frac{1}{2}H_2O$, mp 178—179°, $[\alpha]_D^{29}$ −254° (c=0.871, in MeOH), $[M]^+$ m/e 284.067 (Calcd. 284.068 for $C_{16}H_{12}O_5$), 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° 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.*[3,4] The coexistence of *l*-maackiain and pterocarpin in plant callus tissue is also of interest from chemotaxonomical point of view.

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1) T. Furuya, H. Kojima, and K. Syono, *Chem. Pharm. Bull.* (Tokyo), **14**, 1189 (1966).
2) T. Furuya, H. Kojima, and K. Syono, *Chem. Pharm. Bull.* (Tokyo), **15**, 901 (1967).
3) B. Kaul and E.J. Staba, *Science*, **150**, 1731 (1965).
4) B. Kaul and E.J. Staba, *Planta Medica*, **15**, 145 (1967).