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SHORT COMMUNICATION

ISOLATION OF BERBERINE FROM CALLUS TISSUE OF *COPTIS JAPONICA**

TSUTOMU FURUYA, KUNIHIKO SYŌNO and AKIRA IKUTA
School of Pharmaceutical Sciences, Kitasato University, Minato-ku, Tokyo, Japan

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Abstract—Berberine has been isolated from callus tissue of *Coptis japonica* var *dissecta*

THE DRIED rhizome of *Coptis japonica* Makino var. *dissecta* (Yatabe) Nakai (Seriba-woren in Japanese) (Ranunculaceae) is well-known as a source of berberine and, together with berberine hydrochloride, is widely used as a stomach tonic in Japan. The present paper describes the isolation of berberine from callus tissue of *Coptis* plant

The callus tissue first derived from the petiole of *Coptis* plant was subcultured for about 2 years, collected and extracted with methanol The methanol extract was fractionated to separate the alkaloid fraction, which was submitted to preparative TLC to obtain the crude alkaloid The hydrochloride was recrystallized from water, as yellow needles, m p 192–193° (decomp)[1] and was identical with an authentic sample of berberine hydrochloride by UV, IR and mass spectra This is the first isolation of berberine from a plant callus tissue.

EXPERIMENTAL

Culture condition and extraction procedures The callus derived in August 1968 from the petiole of *Coptis* plant was grown in the dark at 26° on Murashige and Skoog's medium (minus glycine) containing 2,4-D (1 ppm), kinetin (0 1 ppm) and Difco agar (0 9%) and subcultured for about 2 yr at intervals of 6 weeks growth cycle

The callus tissue (fresh weight 64 g, dry weight 3 8 g) was homogenized in Waring blender and extracted with MeOH repeatedly The MeOH extract was acidified with 2 N HCl and shaken with ether The acidic solution was made alkaline with ammonia and extracted with $CHCl_3$ The concentrated $CHCl_3$ extract was submitted to preparative TLC [Silica gel G, 0 25 mm thickness, solvent $CHCl_3$–MeOH (3 1)] The greenish yellow fluorescent zone corresponded to berberine was eluted with ammoniacal MeOH, which was evaporated to dryness After addition of 2 N HCl to the MeOH extract, the crude alkaloid hydrochloride was obtained

Berberine hydrochloride Crude alkaloid hydrochloride was recrystallized from water to give yellow needles, 2 4 mg, m p 192~193° (decomp), UV λ_{max}^{EtOH} nm 231 268, 352, 431, IR ν_{max}^{KBr} cm^{-1} 1603, 1506 (benzene C=C), 1275, 1235, 1034 (C—O—C), mass *m/e* 337 (100%), 336 (81), 322 (68), 321 (90), 320 (71), 307 (37), 306 (42), 292 (37), 278 (83), TLC R_f 0 46 [Silica gel G, solvent $CHCl_3$–MeOH (3 1)] and identified (UV, IR, MS, TLC) by direct comparison with authentic sample of berberine hydrochloride

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[1] H KANEKO, S NARUTO and N IKEDA, *Yakugaku Zasshi* **88**, 235 (1968)

Key Word Index—Coptis japonica, Ranunculaceae, callus tissue, berberine