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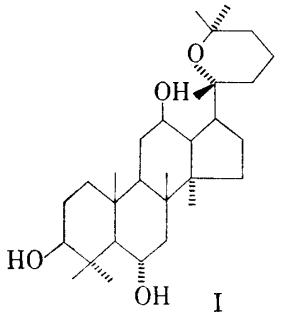
Isolation of Panaxatriol from *Panax ginseng* Callus

Panax ginseng C.A. MEYER (Araliaceae, ginseng) is a perennial herb indigenous to the forests of the eastern Asia and cultivated in Northern China, Korea and Japan. Ginseng root is widely used as a tonics in the Orient from ancient.

Saponins and sapogenins of ginseng were elucidated by Shibata, *et al.*[1] and Elyakov, *et al.*,[2] but that of gineng callus not investigated yet. Now we wish to report the isolation of panaxatriol (I),[1b,2] a genin of ginsenoside Rg_1,[1c,d] in high yield from ginseng callus.

The callus derived from petiole of cultivated ginseng was grown on Murashige and Skoog's agar medium (minus glycine) containing 2,4-dichlorophenoxyacetic acid 1 ppm. The callus has been subcultured at about 25° in the dark and at four to five weeks intervals for about three years.

The callus (500 g fresh weight, 25 g dry weight) harvested was homogenized with cold methanol 650 ml in a Waring blender, refluxed for three hours and filtered. The filtrate was concentrated to a small volume under reduced pressure. To the extract was added cold methanol and the methanol soluble portion was again evaporated to dryness *in vacuo*. Then, the residue was washed with ether. The ether insoluble portion after dissolving water was extracted with *n*-butanol. The butanol layer was evaporated to dryness. The crude saponin (2.8 g) obtained was submitted to thin-layer chromatography on silica gel G (upper layer of *n*-BuOH–AcOH–H_2O=5:1:4) to show almost the same pattern as ginseng saponins. Especially, a large amount of ginsenoside Rg and a small amount of Rb in ginseng callus were detected. The crude saponin was hydrolyzed by refluxing with 5% sulfuric acid in 50% aqueous ethanol. After working up in the usual way, the hydrolysate was put on a silica gel column and gradually eluted with benzene, benzene–ethyl acetate (2:1), and benzene–ethyl acetate (1:1) (one fraction 20 ml). Fraction No. 90—170 gave a crystalline compound, which was recrystallized from



I

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2) *a*) G.B. Elyakov, L.I. Strigina, N.I. Uvarova, V.E. Vaskovsky, A.K. Dzizenko and N.K. Kochetkov, *Tetrahedron Letters*, **1964**, 3591; *b*) G.B. Elyakov, A.K. Dzizenko and Yu. N. Elkin, *ibid.*, **1966**, 141.

ethyl acetate to give colorless needles (I), 23 mg (yield 0.09% per dry weight callus), mp 237—239° and M^+ 476.389 (Calcd. 476.387 for $C_{30}H_{52}O_4$) by high resolution mass spectrometry. I was identical with authentic sample of panaxatriol by mixed melting point test and infrared (IR) spectrum.

It should be noted that ginseng callus contains a high percentage of panaxatriol and the main saponins of ginseng. The isolation of saponins are now in progress.

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