Propolis is a resinous hive product collected by bees. It consists of exudate from plants, which is mixed by bees with beeswax to obtain a sealing material of the required consistency.\(^1\) Propolis balsam, an alcohol extract of propolis, is known to have antioxidative activity.\(^2,3\) This paper reports an antioxidative compound from propolis.

Propolis was obtained from Japanese hives in Gifu, Nagano, and Hokkaido, and imported from China in Hubei and Beijing, from Brazil and United States. Propolis (1.0 g) was extracted with 50 ml of 60% methanol to obtain the balsam. The yields of balsam from Gifu, Nagano, Hokkaido, Hubei, Beijing, Brazil, and United States were 0.34, 0.07, 0.29, 0.42, 0.41, 0.36, and 0.31 g/g of propolis, respectively. The antioxidative activity of propolis balsam was measured by its inhibition of methyl linoleate autoxidation. A mixture of methyl linoleate (294 mg, 1 mmol) and a balsam (73.8 g, 0.025% relative to methyl linoleate) in an ethanol solution was added to a test tube (1.5 cm in diameter), and the solvent was removed in vacuo. Each sample was stored at 60 °C in the dark, before the peroxide concentration was determined by the iodometric method as previously described.\(^4\) Figure 1 shows the effect of each propolis balsam on the autoxidation of methyl linoleate. Each suppressed the autoxidation, the balsam from Beijing exhibiting the most effective inhibition. Therefore, we attempted to isolate the antioxidative components from Beijing propolis.

The balsam from Beijing propolis (2.1 g) was chromatographed in a silica gel (Fuji gel BW-820MH) column (4.5 x 40 cm), the column being developed with an increasing proportion of methanol in chloroform. An antioxidative compound, 1, was isolated from the fraction eluted with 100:3 chloroform–methanol (v/v). Although other fractions also exhibited antioxidative activity, they contained many compounds and further separation was not accomplished.

Compound 1 was crystallized from benzene and obtained as a white powder (0.17 g yield, mp 148–150 °C). The structure of compound 1 was identified to be that of benzyl caffeate (Fig. 2). UV \(\lambda_{	ext{max}}\) (methanol) nm (e): 246 (11,900), 303 (sh. 14,500), 332 (20,100). IR \(\nu_{	ext{max}}\) (KBr) cm\(^{-1}\): 3500 and 3340 (OH), 1695 and 1640 (\(\alpha\)-unsaturated ester carbonyl), 1605 and 1540 (aromatic ring), 1280, 1180, 975 (\(\text{trans} - \text{CH} = \text{CH}\)-). EIMS \(m/z\) (rel. int.) at 70 eV: 270 (M\(^+\), 47%), 252 (8), 225 (22), 179 (7), 163 (43), 161 (17), 136 (31), 107 (11), 91 (benzyl group), 106. \(1^{13} \)C-NMR \(\delta\) (CD\(_3\)OD): 5.04 (2H, broad s, OH), 5.19 (2H, s, H-7), 6.30 (1H, d, J = 15.8 Hz, H-8), 6.79 (1H, d, J = 8.1 Hz, H-5), 6.93 (1H, dd, J = 2.1, 8.1 Hz, H-6), 7.06 (1H, d, J = 2.1 Hz, H-2), 7.26–7.40 (5H, m, H-3, 3′, 4′, 5′, and 6′), 7.58 (1H, d, J = 15.8 Hz, H-7), 13C-NMR \(\delta\) (CD\(_3\)OD): 67.1 (C-7), 114.9 (C-8), 115.2 (C-2), 116.5 (C-5), 123.0 (C-6), 127.7 (C-1), 129.1 and 129.5 (C-2′, 3′, 4′, 5′, and 6′), 137.7 (C-1′), 146.7 (C-3), 147.1 (C-7), 149.5 (C-4′), 169.0 (C-9). The caffeic acid esters in propolis have already been reported as antimicrobial constituents.\(^5,6\)

Caffeic acid and its derivatives are widely distributed in plants,\(^7\) and their outstanding antioxidative activity has been reported.\(^8,9\) Our result indicates that benzyl caffeate (1) also had strong antioxidative activity (Fig. 1).

The amount of benzyl caffeate (1) in each propolis balsam was measured by HPLC. A quantitative HPLC analysis was performed in a \(\mu\)Bondapack \(5 \mu\)C18 column (3.9 x 150 mm, Nikko Waters Co.), using acetonitrile-water-acetic acid (48:50:2, v/v) at a flow rate of 1.0 ml/min. Compounds were detected by their absorbence at 330 nm, benzyl caffeate being eluted at 5.9 min. Table 1 shows the amount of benzyl caffeate in the propolis balsam from each of the seven different regions. The content of benzyl caffeate in each balsam correlated with its antioxidative activity as indicated in Fig. 1.

Flavonoids are the most abundant and most effective antioxidants in propolis balsam.\(^3,10\) This study demonstrates that benzyl caffeate also makes a relatively large contribution to the antioxidative activity of propolis.

### References


