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CAPRIC ACID: A GROWTH INHIBITING SUBSTANCE FROM DORMANT *IRIS HOLLANDICA* BULBS

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Key Word Index—*Iris hollandica*; Iridaceae; iris; growth inhibitor; capric acid; fatty acid; dormancy.

Abstract—A growth inhibiting substance, detected by the *Avena* coleoptile straight growth test, was isolated from dormant iris bulbs and identified as capric acid. Lauric and myristic acids, which have the same inhibitory activity, were also detected.

INTRODUCTION

THE BULBS of Dutch iris, *Iris hollandica* cv. Wedgwood, have about a two month dormant period after the harvest. Judging from changes in the amount of endogenous growth inhibitors in the bulbs using the *Avena* coleoptile straight growth test, growth inhibitors in the acidic and neutral fraction are involved in the dormancy of bulbs. The main acidic inhibitor was identified as abscisic acid using TLC, GLC, and ORD analysis.[1,2] In this paper, we report the isolation and identification of a growth inhibitor in the neutral fraction of Dutch iris bulbs.

RESULTS

Extraction of iris bulbs with MeOH, followed by fractionation with ethyl acetate, column chromatography, and recrystallization of the crude extract, yielded 29 mg of inhibitor, m.p. 29–31°. The IR spectrum showed a characteristic absorption at 2700–2300 and 1715 cm^{-1} for a carboxyl group. The NMR spectrum showed signals at δ 0·86 (3H, *t*), 1·25 (14H, broad *s*), 1·4–1·8 (2H, *m*), and 2·33 (2H, *t*), typical of a saturated fatty acid. Treatment of the inhibitor with diazomethane gave a methyl ester, whose MS exhibited a molecular ion peak at *m/e* 186 and a base peak at *m/e* 74. In addition, prominent peaks were observed at *m/e* 157, 155, 143, 129, 115, 101, and 87.

Based on the above evidence, one of the inhibitors in the neutral fraction of iris bulbs was identified as capric acid. In addition, another eluate from the Sephadex LH20 column contained a small amount of lauric acid and myristic acid, which was identified by GC–MS analysis. These acids had the same inhibitory effects as capric acid on the growth of the *Avena* coleoptile.

Biological activities

The capric acid isolated from iris bulbs and a sample of authentic capric acid had the same inhibitory effect on the growth of *Avena* coleoptile. No additional effects

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derived from impurities were observed. The inhibition, which was induced by lower concentrations (20 and 30 ppm) of capric acid, was reversed by adding IAA (indole acetic acid) (0·1 ppm).

The inhibiting effects of capric acid were compared with those of (±) abscisic acid in the presence of 0·1 ppm IAA. Abscisic acid inhibited the growth of the *Avena* coleoptile at 10^{-7}M, but not perfectly even at 10^{-3}M. Capric acid, however, while showing no effect below 3×10^{-5}M, completely inhibited the growth of the *Avena* coleoptile at 3×10^{-4}M. The same effects were observed in the absence of IAA.

DISCUSSION

Abscisic acid (ABA) is considered to be the main component of inhibitor β, inducing dormancy in seed and buds. Holst[3] pointed out, however, that inhibitor β is much stronger than ABA in the inhibiting effect of buds in potatoes. Lenton *et al.*[4] reported that there is no relation between the level of endogenous abscisic acid and photoperiodically induced bud dormancy. Hasegawa and Hashimoto[5] reported new inhibitors, batatasins, causing the dormancy of yam bulbils. These studies suggest there are some inhibitors inducing dormancy beside ABA, and our own work confirms this.[1,6] The inhibitors in the neutral fraction of iris bulb are capric acid and related compounds, and it is interesting that inhibitory effects of fatty acids on the germination of seed or elongation of lateral shoots have been reported earlier.[7,8]

EXPERIMENTAL

Isolation. In late June 1972, about 1 month after harvest, the dormant *Iris hollandica* bulbs, cv. Wedgwood (97 kg), were homogenized and extracted with MeOH for several weeks at room temp. The filtered extract was evaporated *in vacuo* and the aqueous concentrate, adjusted with HCl to pH 3·0, was extracted with EtOAc. After removing the acidic substances with 2% $NaHCO_3$, the EtOAc fraction was concentrated to a residue (99 g). The residue was applied on a column of silicic acid–celite (1 : 1) which was packed in C_6H_6 in a ratio of 1 g sample per 10 g silicic acid and eluted with C_6H_6, containing increasing amounts of EtOAc. Inhibiting activities were observed in several fractions, but the 10% EtOAc fraction (28·3 g) had the strongest activity. This was rechromatographed on a silicic acid–celite column in $CHCl_3$–C_6H_6. The active fraction (3·5 g) was eluted with C_6H_6 containing 7·5–15% $CHCl_3$. This residue was dissolved in a small amount of $CHCl_3$ and treated with MeOH, which precipitated inactive material. The remaining MeOH-soluble fraction (2·2 g) was applied on a Sephadex LH20 column in a ratio of 1 g sample per 70 g Sephadex and eluted with MeOH in H_2O (50–100%). Inhibiting activity was detected in the effluents containing 65–75% MeOH. These eluates eventually yielded 98 mg of a crude active material. Recrystallization from MeOH–H_2O gave colourless plates (29 mg). The eluate of 75% MeOH, which had several spots on TLC, was methylated with diazomethane for GC–MS analysis.

Bioassay. The *Avena* coleoptile straight growth test[9] was used for determining the growth inhibiting activities. IR spectra were run in $CHCl_3$. NMR spectra (90 MHZ) were recorded in $CDCl_3$ with TMS as an internal standard.

Combined GC–MS. A methyl ester of the inhibitor, prepared with CH_2N_2 was chromatographed on a Hitachi K53 Gas Chromatograph using a 1 m × 3 mm stainless steel column, 10% PG 20 M on 40–60 mesh Celite 545 at 115°. MS were obtained with a Hitachi RMS-4 instrument and the peaks were scanned from *m/e* 1–300 in 3 sec.

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