Lichti and Wartburg postulated[2] a possible long range coupling between C_1-H and C_4-H with J=1.2 c.p.s. (upon harpagoside, harpagide and their derivatives), which stimulated us to perform a double resonance study. According to our decoupling experiment of Ⅳ (at 60 Mc.), contrary to their presentation, a proton coupling to C_4-H with J=*ca.* 1.2 c.p.s. was found to be C_9-H instead of C_1-H (Table I). Thus, upon irradiation at τ 6.80 (C_9-H), a quartet at τ 4.50 (J=6.6 and *ca.* 1.2 c.p.s.) was found to change into a doublet (J=6.6 c.p.s.), together with the sharpening of a broad singlet at τ 3.97 (C_1-H) as expected. This can reasonably be understood in view of a long range coupling across four σ bond.[7]

The glycoside portion other than G-side I (mostly remained in the water soluble part) has now been under study in this laboratory.

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*4 Calculated by means of the integrated peak area due to total aromatic protons and also to two high field aromatic protons adjacent to methoxyl function in O-methyl-*p*-coumaroyl moiety appearing at τ 3.15 (multiplet), which were easily discriminated from other aromatic protons.

*5 All the attempts (chromatography, fractional recrystallization etc.) to separate G-side I or its pentaacetate into the two components were failed.

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Isolation of a Plant Growth Inhibitory Substance from Garden Peas (*Pisum sativum* L.) and Its Identification with (+)-Abscisin-II

The investigation on plant growth regulators has become active recently and the several active substances have been isolated, especially from the higher plants.

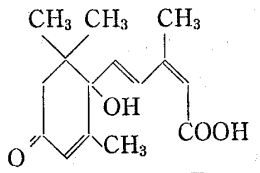
We have been attempting to isolate plant growth regulators from garden peas (*Pisum sativum* L.), using the rice seedlings growth test for bioassay, and found the presence of a gibberellin-like substance and a growth inhibitor. In this communication, we wish to report the isolation of the inhibitor and its identification with (+)-abscisin-Ⅱ.

The fresh garden peas (1300 kg.) were broken into fragments and extracted twice with methanol overnight at room temperature.

The extract was concentrated and the concentrate was extracted with ethyl acetate. The ethyl acetate fraction was concentrated and then extracted with 5% sodium hydron carbonate. The extract was acidified with 2*N* hydrochloric acid and extracted with ethyl acetate.

The removal of ethyl acetate gave 22.20 g. of an acid fraction. This fraction was purified twice by absorption chromatography on an active charcoal-celite (1:2) which was eluted with increasing concentration of acetone in water. The inhibitory activity was found in the 20% and 30% acetone eluates which gave oily material (1.32 g.). This material was further purified by chromatography on silica gel with increasing concentration of methanol in methylene chloride. The active fraction (312 mg.) was found in the eluate of 5% methanol and 95 mg. of this fraction was then rechromatographed on silica gel-Celite (silica gel was pretreated with oxalic acid) with increasing proportion of ethyl acetate in benzene. The inhibitory activity was found in 15% ethyl acetate eluate which afforded 9 mg. of a crystalline substance, whose recrystallization from hexane-ether gave 1.2 mg. of minute platelets, m.p. 167~168°.[1] $[\alpha]_D^{11}$ +488 (c=0.123, EtOH). ORD in ethanol (c=0.0176), 14°: $[\alpha]_{700}$ +341°, $[\alpha]_D$ +488°, $[\alpha]_{289}$ +30,700°, $[\alpha]_{245}$ −85,200°, $[\alpha]_{216}$ +14,200°, and $[\alpha]_{200}$ 0°. UV λ_{max}^{EtOH} mμ: 240 (shoulder, ε=18,800) and 258 (ε=21,000). IR (KBr) cm^{-1}: 3420, 3200~2300, 1675, 1648, 1622, 1600, 978. NMR (60 Mc., $CDCl_3$, τ): 8.96 (3H, singlet), 8.88 (3H, singlet), 8.06 (3H, doublet, J=1.5 c.p.s.), 7.94 (3H, doublet, J=1.5 c.p.s.), 7.90, 7.63, 7.58, 7.31 (2H, AB-type quartet), 4.22 (1H, broad singlet), 4.01 (1H, broad singlet), 3.84 (1H, doublet, J=16 c.p.s.), 2.18 (1H, doublet, J=16 c.p.s.).

In thin-layer chromatography (silica gel treated with oxalic acid was used as the adsorbent, the chromatogram was developed with methanol-methylene chloride (1:9), and the plate sprayed with dilute sulfuric acid and heated was observed), the inhibitory substance (Rf 0.45) gave a light yellow spot with no tailing and a characteristic yellowish green fluorescence under ultraviolet light.



(+)-Abscisin-II

These physical data of the inhibitory substance were identical with those reported for (+)-abscisin-II which was isolated from the young fruit of cotton (*Gossypium hirsutum* L.),[2] the leaves of sycamore (*Acer pseudoplatanus* L.),[3] and the seeds with the pods of lupin (*Lupinus luteus* L.)[4] as a plant growth inhibitory substance and whose chemical structure was determined by physicochemical methods[5] and confirmed by synthesis.[6,7] Further investigation to isolate other active substances from garden peas is now in progress.

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