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Isolation and Structure of Gibberellin A₃₅ and its Glucoside from Immature Seed of *Cytisus scoparius*

Sir:

We wish to report here the isolation and structural determination of a new gibberellin, gibberellin A₃₅ (GA₃₅) and its glucoside from immature seed of yellow broom (*Cytisus scoparius*).

The methanol extract of immature pods of yellow broom (37 kg) was separated into ethyl acetate-soluble and *n*-butanol-soluble acidic fractions by the usual method. Two gibberellin-like substances (F-I and F-II) were isolated from the ethyl-acetate soluble acidic fraction by the following procedures: i) counter-current distribution (1M phosphate buffer of pH 5.42-ethyl acetate, 10 transfers); ii) adsorption chromatography (charcoal, elution with acetone-water; iii) adsorption chromatography (silicic acid, elution with ethyl acetate-benzene). From the fraction of ethyl acetate-benzene (50:50) in iii, F-I (20 mg) was isolated by fractional crystallization. The mother liquor of the recrystallization and the fraction of ethyl acetate-benzene (60:40) in iii) were purified by preparative TLC after methylation with diazomethane to afford the methyl esters of F-I (5 mg) and F-II (6.6 mg).

F-I and F-II¹⁾ showed mp 269~271°C and 248~252°C, respectively. F-I-Me²⁾ and F-II-Me showed the molecular ion peak at *m/e* 362 in their mass spectra, while TMS-F-I-Me²⁾ at *m/e* 434 and TMS-F-II-Me at *m/e* 506. This suggests the molecular formula C₁₉H₂₄O₆ for

F-I and F-II and the presence of a hydroxyl group in F-I and two in F-II. This was further confirmed by the following spectral properties. The IR spectrum of F-I-Me showed the presence of a hydroxyl (3520 cm⁻¹), a γ -lactone (1773) and a methoxycarbonyl (1710) groups, but no double bond. On the other hand, the presence of hydroxyl (3446) and an exocyclic methylene (1653) groups and two carbonyl functions (1730) in F-II was indicated.

The NMR spectrum of F-II-Me in CDCl₃ showed a 3H singlet at τ 8.85 due to the C-4 Me of the gibberellane ring, two 1H broad singlets at τ 4.96 and 5.09 due to the exocyclic methylene protons and a pair of 1H doublets at τ 7.26 and 6.74 ($J=10.5$ cps) characteristic of the C-5, C-6 protons. A 1H triplet at τ 6.14 and a 1H multiplet at τ 5.78 were assigned to two carbinylic protons.

The NMR spectrum of F-I-Me in CDCl₃ exhibited a 3H singlet at τ 8.78 (C-4 Me), a pair of 1H doublets at τ 7.47 and 6.78 ($J=6.5$ cps, C-5, C-6 protons) and a 1H triplet at τ 6.10 (carbinylic proton). A 3H singlet at τ 8.57 is characteristic of the C-16 Me in GA₃ and GA₁₀ type gibberellins and a 1H triplet

at τ 5.47 was considered to be due to $\overset{\text{H}}{\underset{|}{\text{C}}}\text{-OR}$, which is distinctive in its very low τ value.

On the basis of these spectral properties, it was concluded that F-I and F-II contain the functional groups summarized in the Table. The comparison of the functionalities between F-I and F-II suggests the possibility that F-I was derived from F-II by the ether ring formation between one of the hydroxyl groups

1) The pure F-II was obtained by the enzymatic hydrolysis of its glucoside.

2) F-I(II)-Me: methyl ester of F-I(II). TMS-F-I(II)-Me: trimethylsilyl ether of F-I(II)-Me.

TABLE. FUNCTIONAL GROUPS IN F-I AND F-II

	F-I	F-II
sec. OH	1	2
γ -Lactone	1	1
COOH	1	1
>C=CH_2	0	1
$\text{-}\overset{ }{\underset{ }{\text{C}}}\text{-CH}_3$	2	1

and the exocyclic double bond in F-II. This was confirmed by the quantitative conversion of F-II to F-I by boiling F-II in 0.3N methanolic HCl for 1 hr. Such a facile ether formation can be expected only between the C-11 β or C-12 β hydroxyl group and C-16. The possibility for the C-12 β hydroxyl group in F-II was excluded by the observation of no down-field shift³⁾ of the exocyclic methylene protons. When ether bridge is formed between C-11 β and C-16, the conformation of the C-ring should be chair and the dihedral angle between the C-5 and C-6 protons is changed. Thus the very low τ value (τ 5.47) of a 1H triplet due to the C-11 α protons (equatorial) and a small coupling constant of the C-5 and C-6 protons can be explained by this conformational change. The presence of a hydroxyl

group at C-3 β in F-I and F-II was indicated by the down-field shift (0.3 ppm) of the C-4 Me in their NMR spectra in the change of the solvent from CDCl₃ to pyridine-d₅. Thus the structures I and II were assigned to F-II and F-I, respectively.

There remains the possibility for F-I to be an artifact formed from F-II in the purification process. The reexamination by gas chromatography of the fraction containing gibberellins obtained by careful extraction and purification disclosed that F-II is the main gibberellin and the amount of F-I was very small. Since F-I is quite possible to be an artifact, the name of gibberellin A₃₅ was allocated to F-II only.

The *n*-butanol-soluble acidic fraction was purified by the following procedures: i) adsorption chromatography (charcoal, elution with acetone-water); ii) partition chromatography (1M phosphate buffer of pH 5.65 on Sephadex G-50, elution with *n*-butanol-ethyl acetate); iii) adsorption chromatography by gradient method (charcoal, elution with acetone-water); iv) adsorption chromatography (silicic acid, elution with methanol-chloroform); v) preparative paper chromatography (solvent system, isopropanol-7N aq. ammonia-water, 8:1:1). Thus 130mg of a new gibberellin glucoside (F-III) was isolated.

F-III yielded GA₃₅ on cellulase hydrolysis, and glucose and F-I on acid hydrolysis. The NMR spectrum of F-III in CD₃COCD₃-D₂O showed an anomeric proton at τ 5.57 ($J=7$ cps). Thus it must be GA₃₅-glucoside.

F-III afforded a pentaacetate by treatment with pyridine-acetic anhydride. On 0.1N methanolic HCl hydrolysis (boiling, 1 hr), it yielded F-I acetate (IV). This indicates that F-III is 5-*O*- β -D-glucosyl-gibberellin A₃₅ (III). This assignment was further supported by a decoupling experiment which disclosed that the signal due to the C-11 α proton remains in the neighbourhood of τ 5.78 in the NMR spectrum of the pentaacetate in CDCl₃.

In the preliminary bioassay, the growth promoting effect of GA₃₅ on rice seedlings is

3) The compound V, one of the NaBH₄ reduction products of GA₂₆-Me diacetate, shows clear down-field shift of the exocyclic methylene (2H broad singlet, τ 4.84).

evaluated to be about one tenth as much as that of GA_3 and about ten times as much as that of F-I.

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Hisakazu YAMANE
Isomaro YAMAGUCHI
Noboru MUROFUSHI
Nobutaka TAKAHASHI

Department of Agricultural Chemistry
The University of Tokyo
Tokyo

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