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STRUCTURES OF NEW GIBBERELLIN GLUCOSIDES IN IMMATURE SEEDS OF PHARBITIS NIL

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We have isolated seven gibberellin glucosides, tentatively named F-I, F-II F-III, F-IV, F-V, F-VI and F-VII* from immature seeds of the Japanese morning-glory (Pharbitis nil) (1,2) and shown that F-I and F-V are 2-O-β-glucosyl-gibberellin A_3 and 3-O-β-glucosyl-gibberellin A_8 (3), respectively. Here we report the structural elucidation of F-II, F-III, F-IV and F-VI.

On the treatment with acetic anhydride-pyridine at room temparature the four glucosides gave the corresponding acetates. Chemical shifts in the NMR spectra of the glucosides and their acetates are summarized in Table.

Esterification of a crystalline F-II tetraacetate (II), $C_{33}H_{40}O_{15}H_2O$**, m.p. 204-206°, with diazomethane afforded an amorphous dimethyl ester (III), which shows a strong UV absorption (λ_{max} 253 mμ, ε 17,000) due to a heteroannular diene system. No γ-lactonic band is observed in the IR spectrum of F-II. On acid hydrolysis, F-II yielded gibberic acid (4) and glucose exclusively. The above evidences indicate that F-II is composed of each one mole of gibberellenic acid (5) and glucose. The NMR spectrum of III not only supports the above aspect but also indicates the position of the glycosidic linkage. The C-2 proton doublet at τ 5.75 (overlapped with C-6' protons) and the anomeric proton doublet at τ 5.35 (J=7.0 cps) characteristic of β-glucose show no differences in the chemical shifts from those of F-II. This indicates that the glucose moiety is attached to C-2.

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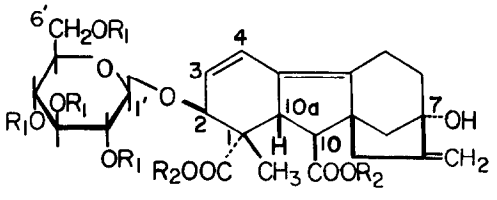
* The structural elucidation of F-VII is in progress.

** Satisfactory analyses were obtained for the compounds whose molecular formulae are listed in this report.

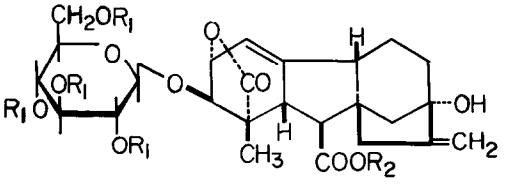
Table. NMR data (τ values) (i) in $CDCl_3$ (ii) in $(CD_3)_2CO$-D_2O (10:1)

Signals* are not distinguishable by overlapping with other signals.

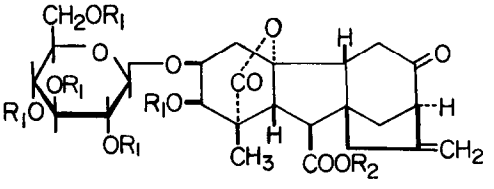
	F-II		F-III	F-IV		F-VI	
Compound	I	III	IV	VI	VII	IX	X
Solvent	(i)	(ii)	(i)	(i)	(ii)	(i)	(ii)
$-CH_3$(C-1)	8.68	8.81	8.76	8.78	8.89	8.75	8.84
H-2	5.73	5.75	5.75	*	4.78	*	4.79
H-3	4.04	4.14	*	*	6.18	*	6.05
H-4	3.73	3.65	4.20	*	*	*	*
H-7				6.83	6.80	*	*
H-10	7.55	7.42	7.35	7.33	7.30	7.46	7.37
H-10a	*	*	6.78	6.62	6.75	7.23	7.26
$>C=CH_2$	5.12	5.02	5.14	4.91	4.99	5.21	5.18
	4.88	4.75	4.95	4.81	4.82	5.10	5.02
$-CH_2OCO-$						*	5.50, 5.85
$-COOCH_3$		6.41, 6.38					
H-1' (anomeric)	5.57	5.35	5.50	5.47	5.53	5.51	5.44
$-OCOCH_3$		8.00, 7.98			8.05, 8.00		8.02, 7.97
		7.94, 7.93			7.98, 7.95		7.95, 7.91
					7.92		7.88



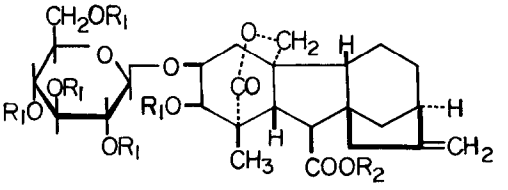
I $R_1=R_2=H$
II $R_1=COCH_3$, $R_2=H$
III $R_1=COCH_3$, $R_2=CH_3$



IV $R_1=R_2=H$
V $R_1=COCH_3$, $R_2=CH_3$



VI $R_1=R_2=H$
VII $R_1=COCH_3$, $R_2=H$
VIII $R_1=COCH_3$, $R_2=CH_3$



IX $R_1=R_2=H$
X $R_1=COCH_3$, $R_2=H$
XI $R_1=COCH_3$, $R_2=CH_3$

Thus the structure I, 2-O-β-glucosyl-gibberellenic acid, is assigned to F-II.

On acid hydrolysis F-III also yielded gibberic acid and glucose exclusively. F-III shows IR bands at 1750 cm^{-1} (γ-lactone) and 1700 cm^{-1} (carboxyl). The NMR spectrum of F-III shows signals characteristic of isogibberellin A_3 (6,7), namely, the C-2 proton doublet at τ 5.75 (J=5.0 cps) and the C-4 proton broad multiplet at τ 4.20 (the C-3 proton signal could not be assigned by overlapping with those of the exomethylene), as well as the β-anomeric proton doublet at τ 5.50 (J=7.0 cps). Successive acetylation and esterification of F-III afforded a monomethyl ester of tetraacetate (V) which still retains a free tertiary hydroxyl (a band at 3500 cm^{-1} in the IR spectrum), suggesting that F-III should have the same type of a glycosidic linkage as in F-II. Now the structure IV, 2-O-β-glucosyl-isogibberellin A_3, can be assigned to F-III.

F-II and F-III are considered to be artifacts derived from F-I, since gibberellin A_3 has been known to give gibberellenic acid readily under a mild acidic condition and isogibberellin A_3 under a mild alkaline condition.

F-IV released gibberellin A_{26} (8) and glucose on acid hydrolysis. F-IV yielded an amorphous pentaacetate (VII) and an amorphous monomethyl ester of VII (VIII), $C_{36}H_{44}O_{17}$, having no free hydroxyl, indicating that F-IV is composed of each one mole of gibberellin A_{26} and glucose. All signals in the NMR spectra of VII and VIII well correspond to the components. The presence of a glycosidic linkage at C-3 is deduced from the following observations. In the NMR spectrum of VII, the C-2 proton doublet at τ 4.78 (partly overlapped with the exomethylene proton signal) and the C-3 proton multiplet at τ 6.18 are observed. The anomeric proton doublet chracteristic of β-glucose also appears at τ 5.53 (J=7.0 cps). The low τ value of the C-2 proton signal is indicative of the presence of an acetoxyl group at C-2 and, therefore, the glucose moiety must be attached to C-3. Thus, the structure of F-IV must be VI, 3-O-β-glucosyl-gibberellin A_{26}.

F-VI was purified through alkali hydrolysis of its crystalline pentaacetate (X), m.p. 234-236°, which was chromatographycally isolated from the crude acetylated F-VI fraction. On enzymatic (emulsin) and acid hydrolyses of F-VI gibberellin A_{27} (8) and glucose were formed. X was converted to a monomethyl ester (XI), $C_{37}H_{48}O_{16}$, m.p. 239-240°, having no free hydroxyl. The NMR spectra of X and XI

indicate that each one mole of gibberellin A_{27} and glucose constitute F-VI. In the spectrum of X the C-2 proton doublet at τ 4.79 (J=4.0 cps), the C-3 proton multiplet at τ 6.05 and the β-anomeric proton doublet at τ 5.44 (J=7.5 cps) are observed. The same consideration on these signals as for F-IV allows to assign the structure IX, 3-O-β-glucosyl-gibberellin A_{27}, to F-VI.

It is noteworthy that gibberellin glucosides, F-IV, F-V and F-VI, each having a glycosidic linkage at C-3, are readily hydrolyzed with emulsin, while F-I, having a glycosidic linkage at C-2, shows resistance to the enzymatic hydrolysis.

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