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GIBBERELLIN IN IMMATURE SEEDS OF PHARBITIS NIL

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The presence of gibberellin-like substances in immature seeds of morning-glory (Pharbitis nil) has been suggested by Ogawa (1,2), Murakami (3) and Zeevaat (4). Now we wish to report the isolation and structure of a new gibberellin, tentatively named Pharbitis Gibberellin, as well as the presence of gibberellin A_3 (GA_3) in the seeds.

The ethyl acetate-soluble acidic fraction obtained from 60 kg of immature seeds was successively subjected to ten transfers counter-current distribution method and charcoal chromatography. Elution with acetone-water mixtures, 50:50 and 70:30, gave active fractions, F-I and F-II, respectively. Histogram obtained from thin layer chromatography* and bioassay revealed that different active principles are contained in F-I and F-II. Each fraction was purified through successive procedures: silicic acid adsorption, partition and preparative thin layer chromatography. F-I thus purified showed a fluorescent spot** at R_{GA_3} 1.00 on thin layer chromatogram without heating. This spot also exhibited marked growth promoting activity to dwarf maize mutant d-5. When methyl ester of pure F-I was subjected to thin layer chromatography, it revealed a fluorescent spot, whose R_f value was identical with that of GA_3 methyl ester. Thus, the presence of GA_3 in the seeds was established.

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* Solvent system: for acidic fraction, benzene-n-butanol-acetic acid (70:25:5, v/v). Adsorbent: Silica gel G.

** When plates are sprayed with 70 % (v/v) sulfuric acid and heated at 100°, all known gibberellins give blue or green fluorescent spots under ultraviolet light. GA_3 and GA_7 give fluorescence without heating.

Purified F-II showed a spot at R_{GA_3} 1.60 on thin layer chromatogram. This spot exhibited growth promoting activity to dwarf maize mutant d-5, while rather weak activity to rice seedling. Althogh the active principle could not be obtained as crystals in this stage, preparative thin layer chromatography of its methyl ester afforded 7 mg of colorless crystals, m.p. 181°, which showed a fluorescent spot typical of gibberellins at $R_{GA_3\text{-Me}}$ 1.55 on usual treatment of a thin layer plate. Its IR spectrum is different from those of known gibberellin methyl esters, indicating that it should be a new gibberellin methyl ester.

High resolution mass spectrum* of this ester revealed a parent ion peak at m/e 346 with composition $C_{20}H_{26}O_5$. On the basis of IR and NMR** spectra, the presence of functional groups illustrated in Table I was assumed.

TABLE I

		IR, cm^{-1} (nujol)	NMR, ($CDCl_3$)
1	OH	3520	
1	lactone	1778	
1	$COOCH_3$	1720	6.33 (3H s)
1	double bond (exocyclic methylene)	1670, 882	4.80 (1H s) 5.10 (1H s)
1	$-C-CH_3$		8.93 (3H s)

Since this ester is a mono-methyl ester, the original acid must be a mono-basic acid having molecular formula $C_{19}H_{24}O_5$. Thus, the second active principle in the seeds has been proved to be a new C_{19} gibberellin and we tentatively named it Pharbitis Gibberellin (PG).

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* High resolution mass spectrum was measured by a JNS-01S spectrometer with direct inlet system, electron accelerating voltage being 40 eV.

** NMR spectra were measured by a JNM-4H-100 spectrometer at 100 mc.

In the high resolution mass spectrum, PG methyl ester exhibited prominent peaks due to M-32(CH_4O), M-46(CH_2O_2), M-60($C_2H_4O_2$), M-78($C_2H_6O_3$), M-104($C_3H_4O_4$), M-106($C_3H_6O_4$) and M-122($C_3H_6O_5$) fragment ions, together with $C_{17}H_{17-21}$ hydrocarbon ion peaks, indicating that PG retains the common structural feature to C_{19} gibberellins (5). Intensity of $C_{17}H_{19-23}O$ ion peaks is stronger than that of corresponding hydrocarbon ion peaks. This suggests that a hydroxyl group is located at C-7.

According to Hanson's information (6) concerning NMR spectra of C_{19} gibberellin methyl esters, the spectrum of PG methyl ester (in $CDCl_3$) was compared with that in pyridine in details. The C-1 methyl singlet appeared at τ8.93 in $CDCl_3$ and τ8.85 in pyridine. Since deshielding effect in the pyridine solution was not observed, a hydroxyl group can not be present at C-2. This is concordant with the presence of an AB quartet due to C-10a and C-10 hydrogens, which is characteristic of the gibbane skeleton, at rather high field, τ7.33, 7.50 in $CDCl_3$ and τ7.10, 7.30 in pyridine. Two 1H singlets due to the C-8 exocyclic methylene exist at τ5.10 and 4.80 in $CDCl_3$. In the pyridine solution, this signal shifts to 4.94 and 4.42. This deshielding effect to one of the exocyclic methylene protons can be explained by the presence of a hydroxyl group at C-7.

These physico-chemical evidences allow to assign the structure I (R=H) to PG.

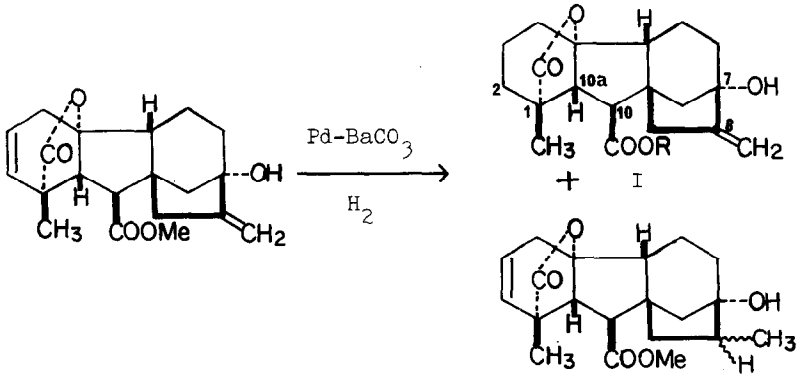

FIG. I

Further, this conclusion was confirmed through chemical conversion of GA_5 methyl ester into PG methyl ester by partial hydrogenation. Catalytic hydrogenation of GA_5 methyl ester using partially poisoned palladium-barium carbonate catalyst (7) afforded a 3:1 mixture of I ($R=CH_3$) and II, which were successfully separated from each other through adsorption chromatography of silicic acid impregnated with silver nitrate. I ($R=CH_3$), thus obtained, was completely identical with PG methyl ester in all respects, IR, NMR and melting point.

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