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STRUCTURES OF NEW GIBBERELLINS IN IMMATURE SEEDS OF CANAVALIA GLADIATA

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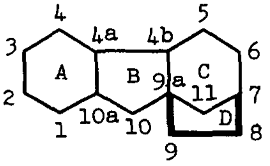
Isolation and biological activities of two novel gibberellins, Canavalia gibberellin-I and -II (CG-I, CG-II) contained in immature seeds of sword bean (Canavalia gladiata DC.) have been already reported (1). In this paper we wish to report the determination of their structures.

On treatment with diazomethane CG-I (m.p. 244-246°) affords a methyl ester (m.p. 161-162°). The high resolution mass spectrum of the ester reveals a molecular ion peak at m/e 390.164, and elemental composition analysis assigns the molecular formula to $C_{21}H_{26}O_7$. Two 3H singlets due to two methoxycarbonyls in the NMR spectrum show the ester to be a dimethyl ester, and the molecular formula, $C_{19}H_{22}O_7$, is assigned to the original acid. Presence of gibbane skeleton (I), common to all known gibberellins, is suggested in the ester by appearance of M-32(CH_4O) and M-60($C_2H_4O_2$) peaks in the high resolution mass spectrum (2) as well as by observation of an AB quartet due to C-10 and C-10a protons in the NMR spectrum. Further, the presence of functional groups illustrated in TABLE 1 is assumed on the basis of the IR and NMR spectra. Anomolously the NMR spectrum of the ester lacks a 3H singlet due to C-1 methyl characteristic of the known gibberellins and reveals two 3H singlets due to methoxycarbonyls, suggesting that CG-I contains a carboxyl instead of a methyl at C-1. In the high resolution mass spectrum of the methyl ester the hydrocarbon fragments with the largest carbon number consist of sixteen carbons, whereas those of seventeen carbons are



I

TABLE 1. Functional groups in CG-I methyl-ester.

	IR, cm^{-1} (nujol)	NMR, τ ($CDCl_3$)
1 OH	3540	
1 γ-lactone	1785	
2 $COOCH_3$	1740, 1726	6.34 (3H, s), 6.36 (3H, s)
1 double bond (exomethylene)	1756, 880	4.80 (1H, s), 5.11 (1H, s)
No $-\overset{|}{\underset{|}{C}}-CH_3$		

usually observed in the case of the methyl ester of known C_{19} gibberellins (2). On acid treatment CG-I gave three acidic compounds, III, IV and V, with evolution of 0.3 mole of carbon dioxide. Since the quantity of each product was too small to be availed for characterization and structural elucidation, its structure was deduced on the basis of IR, NMR and UV spectra of the methyl ester. All of them contain <u>tert</u>.-methyl (NMR: τ8.95-9.00, 3H, s) but neither hydroxyl nor exomethylene (IR), suggesting the occurrence of Wagner-Meerwein rearrangement of rings C/D in CG-I. III must be a simple rearrangement product because it is dibasic and contains a γ-lactone. As shown below IV is a tri-

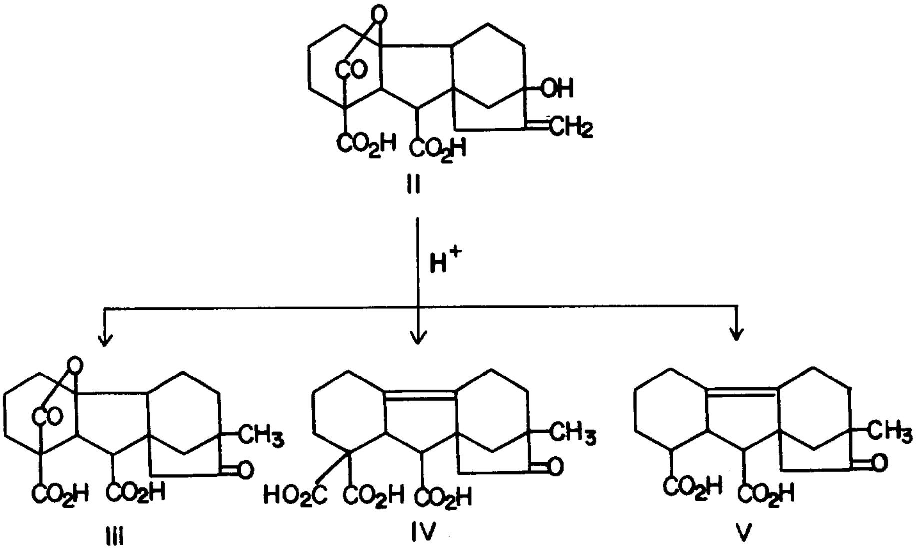


FIG. 1.

carboxylic acid without the γ-lactone. V is a dicarboxylic acid without the γ-lactone, suggesting that the lactone group in II has been decarboxylated. The presence of a tetrasubstituted ethylenic double bond in IV and V was confirmed by UV spectra of their esters ($\lambda_{max.}$ 204-208 mμ, ε 30000) and its location was assumed to C-4a~C-4b through the appearance of the signal due to C-10a proton in the NMR spectra of the esters. Formation of V indicates that the carboxyl and lactone carbonyls at C-1 are located in 1, 3-relationship. The presence of C-7 hydroxyl and C-8 exomethylene groups in CG-I has been suggested by the rearrangement. This is further supported by the large deshielding of one of C-8 exomethylene protons in NMR spectrum measured in pyridine (3) as well as by the occurrence of $C_{16}H_{16-23}O$ peaks which are more intense than $C_{16}H_{16-23}$ peaks in the high resolution mass spectrum (2). The structure II for CG-I accomodates all evidences above cited.

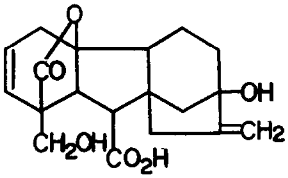
In the same way as in the case of CG-I, the molecular formulas, $C_{19}H_{22}O_6$ and $C_{20}H_{24}O_6$, are assinged to CG-II (m.p. 213-214°) and its methyl ester (m.p. 171-172°), respectively, and the presence of gibbane skeleton is confirmed. The functional groups in CG-II methyl ester are illustrated in TABLE 2. In the NMR spectrum of the ester the C-1 methyl signal can not be observed,

TABLE 2. Functional groups in CG-II methyl ester.

	IR, cm^{-1} (nujol)	NMR, τ ($CDCl_3$)
2 OH	3320, 3560	
1 γ-lactone	1760	
1 $COOCH_3$	1740	6.32 (3H, s)
2 double bonds	1730, 1755	4.15 (2H, s)
(exomethylene)	870	4.78 (1H, s), 5.12 (1H, s)
1 $-\overset{|}{\underset{|}{C}}-CH_2OH$		6.24 (2H, s)
No $-\overset{|}{\underset{|}{C}}-CH_3$		

whereas the presence of a hydroxymethyl group is indicated by a 2H singlet at τ6.24 in $CDCl_3$ as well as an AB quartet at τ5.69, 5.91 in pyridine. This suggests that in CG-II a hydroxymethyl is attached to C-1 instead of methyl.

In high resolution mass spectrum of the methyl ester the intense peaks, M-32 (CH_4O), M-60($C_2H_4O_2$), M-62(CH_2O_3), M-78($C_2H_6O_3$), M-90($C_3H_6O_3$), M-122($C_3H_6O_5$) and M-135($C_4H_7O_5$), are observed. Among them M-135 peak can be ascribable to eliminations of $C_2H_4O_2$ from C-10 methoxycarbonyl, CO_2 from lactone and CH_2OH from C-1. The position of another hydroxyl is assigned to C-7 by large deshielding of one of C-8 methylene protons in the NMR spectrum measured in pyridine and occurrence in the high resolution mass spectrum of $C_{17}H_{16-20}O$ peaks more intense than $C_{17}H_{16-20}$ peaks. The presence of a double bond other than the C-8 exomethylene is shown by a 2H singlet at τ4.15 in the NMR spectrum measured in $CDCl_3$. In deuteroacetone this signal splits into a complex pattern which is quite similar to that of gibberellin A_5 methyl ester, suggesting the presence of C-2~C-3 double bond in CG-II. Further, the methyl ester shows UV absorption at 223 mμ (shoulder, ε 1000) characteristic of the gibberellin which contains C-2~C-3 double bond (4). Thus, the structure VI has been proposed to CG-II.



VI

The presence of novel gibberellins containing C-1 substituents with oxidized forms instead of the methyl group has been now confirmed. They may suggest the mode of catabolism of C_{19} gibberellins in higher plants.

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