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GIBBERELLINS OF *ENHYDRA FLUCTUANS*

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Key Word Index—*Enhydra fluctuans*; Compositae; gibberellins A_9 and A_{13}; comparative biological activity.

Abstract—Gibberellins A_9 and A_{13} were isolated from *Enhydra fluctuans* and identified on the basis of m.p., m.m.p., IR MS and co-chromatography.

In continuation of our research on growth regulators from tropical plants,[1–3] we now report the isolation of two gibberellins from the whole plant of *E. fluctuans* (compositae) and their characterization and biological activity. The presence of number of kaurene derivatives in *E. fluctuans* was reported recently by Pakrashi *et al.*[4–6] These observations led us to investigate the nature of endogenous gibberellins present in the plant.

Chromatography of the acid fraction of the methanolic extract of the defatted plant material gave an amorphous solid, m.p. 188–191°, which was a mixture. Repeated chromatography over silica gel, with IR monitoring, afforded two compounds which yielded crystalline solids m.p. 207–209° (A) and 190–194° (B).

Table 1. Comparative potency of GA_9 and GA_{13} isolated from *E. fluctuans*, with that of GA_3 as standard

Gibberellins	Lettuce hypocotyl test[8]	Cucumber hypocotyl test[9,10]	Rice second leaf sheath test[11,12]
GA_9	3/5th of GA_3	10 times more active than GA_3	Almost inactive
GA_{13}	Inactive	1/10th of GA_3	1/10th of GA_3

Compound A was homogeneous on TLC and gave a purple UV fluorescent spot at R_f 0·75 (in EtOAc–$CHCl_3$–HOAc, 15:1:1), after spraying with 5% H_2SO_4 in EtOH followed by heating at 120° for 5 min. The compound showed IR absorption at 3100 (OH of CO_2H), 1760 (γ-lactone), 1720 (CO_2H) 1660 and 895 cm^{-1} (=CH_2) (Lit. value: 3098, 1740, 1723, 1659 and 893 cm^{-1}). The MS of its methyl ester showed a molecular ion peak at m/e 330 and the other peaks were observed at m/e 298, 284, 260, 252, 226, 224 and 208 which are in accord with the peaks of GA_9 methyl ester reported earlier.[7] Finally the

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identity of A with GA_9 was established from its m.p., m.m.p., superimposable IR spectrum with authentic GA_9 and co-chromatography with the authentic sample.

Compound B was found homogeneous in TLC (R_f 0·44; same solvent as above). The IR spectrum of the compound showed peaks ν_{max}^{Nujol} 3500, 1720–1700 and 1660 cm^{-1} (Lit. value: 3500, 1723–1700 and 1600 cm^{-1}). The MS of its methyl ester showed the molecular ion peak at m/e 420. In addition to molecular ion peak there are peaks at m/e 402, 388, 360, 328 and 300 similar to the peaks of authentic GA_{13} methyl ester.[7] Here also the identity of the compound B with GA_{13} was established from its m.p., m.m.p., co-chromatography with authentic GA_{13} and by superimposable IR spectrum with authentic sample.

The results of bioassay of both compounds are presented in Table 1. Comparative potency of different gibberellins was also studied earlier.[13,14] It is evident that GA_9 is more active than GA_{13}. The biological activities[15] of GA_{13} and GA_{17} were compared with our isolated GA_{13} and the results were similar to the published data. This is the first report of the isolation of GA_{13} from higher plant.

EXPERIMENTAL

Isolation of gibberellins. 10 kg whole plant of *E. fluctuans* after defatting with light petrol. was extracted with MeOH. Methanolic extract was concentrated and the residue in ethereal solution was separated into acid and neutral fractions. The acid fraction was chromatographed over silica gel. The column was eluted successively with light petrol., different mixtures of light petrol.–EtOAc (1:1, 1:2; 1:3; 1:4; 1:5; 1:6; 1:7; 1:8; 1:9) and finally with EtOAc. From the light petrol.–EtOAc (1:7) fraction, colourless residue of GA_9 was obtained which was crystallized from acetone–light petrol. in needles m.p. 207–209° (yield 9 mg) (Lit. m.p. 208–211°). From the light petrol.–EtOAc (1:9) fraction amorphous solid of GA_{13} was obtained which was crystallized from the mixture of light petrol.–EtOAc having m.p. 190–194° (yield 12 mg) (Lit. m.p. 194–96°).

Methyl ester of GA_9 and GA_{13}. Both the compounds were converted to methyl esters by treating the methanolic solution of the sample with excess of ethereal CH_2N_2 at 0° for 24 hr. GA_9 methyl ester was purified through column chromatography and crystallized from aq. MeOH, m.p. 135–136° (Lit. value 136°). GA_{13} methyl ester was also purified by the same procedure and crystallized from light petrol.–$CHCl_3$, m.p. 115–117° (Lit. value 117–119°).

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