Identification of Gibberellin A_{20}, Abscisic Acid, and Phaseic Acid from Flowering *Bryophyllum daigremontianum* by Combined Gas Chromatography-Mass Spectrometry

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*Summary.* The presence of abscisic and phaseic acid in a purified acidic extract from flowering plants of the long-short-day plant *Bryophyllum daigremontianum* [(R. Hamet and Perr.) Berg.] was conclusively established by combined gas chromatography-mass spectrometry (GC-MS) of their methyl esters. Gibberellin A_{20} (GA_{20}) was identified by GC-MS of the methyl ester and the trimethylsilyl ether of the methyl ester. The following levels of the 3 compounds per kg fresh weight were estimated: Abscisic acid, 5.5 µg; phaseic acid, 9.4 µg; gibberellin A_{20}, 0.8 µg. When GA_{20} and four other GAs were applied to *Bryophyllum* under short-day conditions, the order of effectiveness for induction of flower formation was: GA_{3} > GA_{1} > GA_{5} = GA_{7} > GA_{20}. The low biological activity of the native GA_{20} is discussed.

*Introduction*

Acidic extracts of the long-short-day plant *Bryophyllum daigremontianum* [(R. Hamet and Perr.) Berg.] contain two gibberellins (GAs), one with GA_{2}, the other with GA_{5}-like properties (Zeevaart, 1969b, c). The level of the GA_{5}-like material increased considerably when flower formation was induced by the shift from long-day (LD) to short-day (SD) conditions, and reached a maximum at the time flower primordia became visible ca. 35 days after the start of SD treatment (Zeevaart, 1969c). Since a high level of GA in the leaves of this species is a prerequisite for production of the floral stimulus (Zeevaart, 1969c), it was of interest to identify the endogenous GAs. We report here the identification of the GA_{5}-like material as GA_{20}, as well as the presence of abscisic acid (ABA) and phaseic acid in a partially purified extract.
prepared from flowering *Bryophyllum* plants. Furthermore, the biological activity of the native GA$_{30}$ applied to *Bryophyllum* under SD is compared with that of several other GAs.

**Materials and Methods**

**Plant Material**

Plants of *Bryophyllum daigremontianum* [(R. Hamet and Perr.) Berg.] were grown in a greenhouse under a 20-hr photoperiod as described previously (Zeevaart, 1969a). In order to induce flower formation, plants which had developed at least 20 leaf pairs, were transferred from the greenhouse to SD conditions in a growth room with 8 h light from fluorescent and incandescent lamps (intensity 3000 fc at 23°C, and 16 h of darkness at 15°C. Various groups of plants were harvested towards the end of the light period after exposure to 30–44 SD when flower primordia were macroscopically visible. The shoot tips and 3–4 uppermost leaf pairs with their axillary shoots were harvested, frozen in liquid N$_2$, and lyophilized. A total of 922 g dry material (ca. 16 kg fresh weight) were collected from 197 plants.

**Application of GAs**

GAs were injected into the 4th leaf pair counted from the tip with the aid of a 100-µl Hamilton syringe as described previously (Zeevaart, 1969a). Leaf pairs 2 and 3 were removed. Floral stages (0 = vegetative, 6 = normal inflorescence) were assigned as before (Zeevaart, 1969b).

**Extraction and Purification Procedures**

The dry material was extracted in 50-g batches and the acidic fraction from each batch was prepared separately following the procedures described for spinach (Zeevaart, 1971). Further purification of the acidic material took place in 4 successive steps:

1. **Charcoal Chromatography.** The ethyl acetate containing the acidic fraction was reduced *in vacuo* to a small volume, 20 ml of distilled water was added, and the rest of the organic solvent evaporated. The aqueous residue was pipetted on top of a charcoal-celite 535 (1:2, w/w) column and eluted with 80% acetone (Zeevaart, 1971).

2. **Silicic-acid Adsorption Chromatography.** The acidic fraction obtained in step 1 was next run *via* a silicic acid-celite 535 (1:2, w/w) column using 2.5 g of silicic acid (Mallinckrodt, St. Louis, Mo., U.S.A., 100 mesh) for 50 g of dry material extracted. The acidic fraction was eluted with ethyl acetate-chloroform (1:1, v/v).

3. **PVP Chromatography.** Insoluble polyvinylpyrrolidone (PVP) obtained from the GAF Corp., New York, N.Y., U.S.A., under the brand name Polyclear AT, was screened (100-mesh sieve) and washed with 0.1 M phosphate buffer at pH 8.0. The washed PVP was slurried in the same buffer to set up a 30 × 1.9 cm column (Glenn et al., 1972).

All acidic material obtained in step 2 was pooled, taken to dryness, and redissolved in 5 ml 0.5 M phosphate buffer at pH 8.0. The acidic fraction dissolved in buffer was pipetted on top of the PVP column and eluted with 0.1 M phosphate buffer (pH 8.0). The first 50 ml of effluent were discarded; the next 200 ml were collected (Glenn et al., 1972). After lowering the pH of the buffer to 2.5 with 6 N HCl, the acidic fraction was prepared by partitioning 5 times with half the volume of ethyl acetate.