Rapid Paper

Identification of Gibberellins A₁₉ and A₂₀ in Vegetative Tissue of Apple

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Gibberellins A₁₉ and A₂₀ were identified in the vegetative tissue (leaf and bud) of apple (*Malus domestica* Borkh.) by sequential Si gel partition column chromatography→reversed phase C₁₈ high performance liquid chromatography (HPLC)→gas chromatography-selected ion monitoring (GC/SIM) or gas chromatography-mass spectrometry (GC/MS) of the biologically active fractions from HPLC. Trace amounts of gibberellins A₁ and A₉ were also observed (based on GC/SIM of the biologically active fraction after the Si gel→HPLC sequence) in vegetative tissues.

Ramirez and Hoad have identified the gibberellins (GAs), GA₁, GA₄, GA₉, GA₁₂, GA₁₅, GA₁₇, GA₂₀, GA₄₄, GA₅₃, iso-GA₇, GA₆₃ and GA₆₈, in extracts of immature seeds of apple (Cox's Orange Pippin) by GC/MS.^{1,2)} Kirkwood and MacMillan³⁾ have also detected GA₆₁ and GA₆₂ in immature apple seed by GC/MS. GA₃ in large amounts, with GA₁ in lesser amounts, has recently⁴⁾ been detected by GC/MS in very immature (*ca.* 4 weeks after full bloom) apple seed of cv. Sunset and Cox's Orange Pippin. Also, in cv. Sunset at 12 weeks after full bloom the following additional GAs have been characterized [GA₇, GA₈, GA₃₅, GA₅₄, 3-epi-GA₅₄, dehydro GA₄, 16,17-

dihydrodihydroxy-GA₄ and 16,17-dihydrodihydroxy-GA₉⁴⁾]. In the vegetative tissue of apple, the presence of GA₁, GA₄, GA₁₉- and GA₂₀- like substances has been noted using Si gel partition chromatography (bioassay detection),^{5,6)} and the presence of a GA₁₉-like substance in the vegetative tissue of apple was more definitely shown after the Si gel partition column→HPLC (bioassay detection) sequence.⁶⁾ In the present paper, GA₁₉ and GA₂₀ were characterized from extracts of the leaf and bud tissues of apple (*Malus domestica* (Borkh.)) using sequential Si gel partition column chromatography→bioassay→HPLC→bioassay→GC/SIM or GC/MS. Also, trace amounts of GA₁ and GA₉ were observed by GC/SIM from the same tissues.

RESULTS AND DISCUSSION

The biologically active fractions 9~16 (GA₁/3/19-like) from a number of Si gel par-

FIG. 1. Qualitative Spectrum after Reversed Phase C₁₈ HPLC of Biological Activity (Measured in Serial Dilution on Dwarf Rice cv. Tan-ginbozu) Originating from (A) Fractions 9~16 (GA₁/3/19-like Compounds) and (B) Fractions 2~8 (GA₉/4/7/20-like Compounds) Collected from a Number of Si Gel Partition Columns of Acidic, Ethyl Acetate-soluble Extracts from Vegetative Leaf and Bud Tissue of Jonathan and McIntosh Apple (Including Two McIntosh Sports).

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tition columns were combined and further purified by HPLC (Fig. 1A). One biologically active peak occurred at t_R 45~48 min (Fig. 1A) which corresponded to the t_R of GA₁₉. A smaller biologically active peak was observed at t_R 24~27 min (Fig. 1A) which corresponded to the t_R of GA₁. These active fractions were further analyzed by GC-SIM after conversion to the TMSi ether of the Me ester. The fragment ions at m/z 462 (M^+), 447 ($M-15$), 434 ($M-28$), 431 ($M-31$), 402 ($M-60$) and 374 ($M-88$), which are characteristic of GA₁₉-Me-TMSi, were selected for ion monitoring of the GA₁₉-like HPLC fraction. As shown in Table I, the fragment ion peaks appeared at the t_R of GA₁₉-Me-TMSi (18.8 min) on capillary GC with the same relative intensity of GA₁₉-Me-TMSi. However, when the fragment ions at m/z 506 (M^+), 491 ($M-15$), 472 ($M-29$), 465 ($M-41$), 448 ($M-48$) and 375 ($M-131$), which are characteristic of GA₁-Me-TMSi, were monitored, there was only a trace of GA₁ (t_R 19.5 min which was the same as the t_R of standard GA₁-Me-TMSi) at each m/z .

The biologically active fractions 2~8 (GA₉/4/7/20-like) from a number of Si gel

partition columns were further purified by HPLC, yielding two major biologically active peaks (t_R 42~57 min, Fig. 1B). One peak occurred at t_R 42~45 min (Fig. 1B), corresponding to the t_R of GA₂₀, and an additional peak was found at t_R 48~57 min (Fig. 1B), which corresponded to the t_R of GA₄, GA₇ and GA₉. These active fractions were further analyzed by GC/SIM as noted above. The fragment ions at m/z 418 (M^+), 403 ($M-15$), 390 ($M-28$), 375 ($M-43$), 359 ($M-59$) and 301 ($M-117$), which are characteristic of GA₂₀-Me-TMSi, were selected for ion monitoring of the GA₂₀-like fraction. Fragment ion peaks were observed at t_R of GA₂₀-Me-TMSi (16.6 min) with the same relative intensity as that of GA₂₀-Me-TMSi (shown in Table I). A full mass spectrum of a compound occurring at the same t_R as GA₂₀ (21 min) on a GC/MS was also obtained from a GA₂₀-like fraction purified and converted as above. The relative intensities of the major ions of the GA₂₀-like compound in the full spectrum were the same as those of GA₂₀-Me-TMSi (Table II). The GA₄/GA₇/GA₉-like fraction was monitored at fragment ions which were characteristic for GA₄ and GA₇, but no

TABLE I. GC/SIM RELATIVE INTENSITY DATA FROM APPLE VEGETATIVE TISSUE EXTRACTS OF CV. MCINTOSH AND JONATHAN, AND AUTHENTIC GA₁₉ AND GA₂₀

GA	Retention time on GC/SIM	Peaks in SIM spectrum with relative abundance in parentheses (m/z values)
GA ₁₉ -like	18.8 min	462 (M^+ , 7)
GA ₁₉ -Me-TMSi	18.8 min	447 (3) 434 (69) 431 (6) 402 (19) 373 (72)
GA ₂₀ -like	16.6 min	447 (4) 434 (62) 431 (7) 402 (22) 373 (38)
GA ₂₀ -Me-TMSi	16.6 min	403 (29) 387 (3) 375 (190) 359 (30), 301 (52)
		403 (32), 387 (6) 375 (188) 359 (37) 301 (50)

TABLE II. GC/MS RELATIVE INTENSITY OF MAJOR IONS IN A GA₂₀-LIKE COMPOUND FROM APPLE (MCINTOSH) AND AUTHENTIC GA₂₀

GA	Retention time on GC/MS	Major ions with relative intensities in parentheses (m/z values)
GA ₂₀ -like	21.10 min	418 (100) 375 (52.2) 207 (17.3)
		403 (7.0) 359 (8.9)
		387 (0.0) 301 (23.8)
GA ₂₀ -Me-TMSi	21.15 min	418 (100) 375 (51.1) 207 (39.8)
		403 (16.6) 359 (14.2)
		387 (2.5) 301 (16.6)

positive indication of the presence of these two GAs was obtained. However, when this fraction was monitored at m/z 330 (M^+), 298 ($M-32$), 284 ($M-46$), 270 ($M-60$), 243 ($M-87$) and 226 ($M-104$), which are characteristic of GA₉-Me, a trace of GA₉ was found (t_R 14.5 min which was the same as the t_R of standard GA₉-Me) at each m/z . Thus GA₁₉ and GA₂₀ were the major GAs characterized from leaf and bud tissue harvested during periods of relatively rapid growth. An unknown, biologically active GA-like substance (t_R 48~51 min) could not be characterized by more definitive means. GA₁ and GA₉ are present as minor components in these vegetative tissues. Approximate estimates (from four replicates of McIntosh tissue, based on a bioassay at the Si gel partition stage of purification) of the contents of GA₁₉ range from 5 to 187 ng/g d.w., and for GA₂₀ range from 18 to 248 ng/g d.w., the ranges depending on the cultivar, as well as on the replicate.

EXPERIMENTAL

Plant materials

McIntosh. Shoot tips from actively growing 11-year-old McIntosh, MacSpur and Morspur McIntosh trees were collected on May 27, 1980 at Summerland, B.C. The tissue distal to and including the leaf and node above the first fully expanded leaf was excised, frozen immediately in liquid N₂, freeze dried and stored in a sealed vial at -20°C. Each sample consisted of ten shoot tips from a single tree and each cultivar was replicated four times. Each shoot sampled arose from a lateral bud on a vigorous one-year-old shoot pruned to 50% of its original length in March of 1980. This procedure insured active early-season growth of the selected buds (shoots).

Jonathan. One year-old appled stem tissue was harvested (December–February) from apple trees grown in a lath house at the Waite Agriculture Research Institute, Adelaide, Australia under controlled nutrient culture.⁷⁾ The tissue was immediately frozen in liquid N₂ and then freeze-dried. Lyophilized samples were ground in a laboratory grain mill, then sent to Calgary sealed in polyethylene with a silica dessicant and stored at -20°C

Extraction and analytical methods. Lyophilized tissues of apple leaves and buds from cv. Jonathan, McIntosh and two McIntosh sports (MacSpur and Morspur McIntosh) were extracted as described by Taylor *et al.*⁸⁾

The acidic, EtOAc fraction was purified on columns of polyvinylpyrrolidone⁹⁾ and charcoal–celite (1:1) eluted with acetone: water (80:20).¹⁰⁾ This was followed by chromatography on a gradient-eluted Si gel partition column¹¹⁾ with detection by the micro-drop bioassay, in serial dilution, on dwarf rice (cv. Tan-ginbozu).¹²⁾ The biologically active fractions were combined and further purified on reversed phase C18 HPLC with detection by bioassay (as above). The biologically active HPLC fractions were analyzed by GC/SIM or GC/MS after conversion to the methyl ester, or methyl ester TMSi ether, using ethereal diazomethane and TRI-SIL (Pierce Chemical Company).

HPLC conditions. A Waters Assoc. ALC/GPL R-401 liquid chromatograph with two Model 6000 pumps, a Waters Assoc. Automated Gradient Controller and a Model U6K universal injector were used. Column: 300 × 7.8 mm i.d. C18 μ -Bondapak, 32.5% MeOH in 1% aq. HOAc at a 0~20 min linear gradient from 32.5% MeOH in 1% aq. HOAc to 73% MeOH in 1% aq. HOAc, with a 20~45 min flow rate at 2 ml/min.

GC/SIM conditions. A Hewlett Packard 5792A and a 5790A Series Mass Selective Detector were used. Column: crosslinked 5% phenylmethyl silicone, 25 m length, 0.33 μ m film thickness, 0.2 mm int. diameter, 10 psi head pressure, 1400 E.M. volts, 60°C initial temp. 270°C final temp., and a programme rate of 24°C/min.

GC/MS conditions. A kratos MS80 RFA GC/MS system with a capillary interface was used. Column liquid phase DB-1, 30 m length, 1 μ m film thickness, 0.322 mm int. diameter, 60°C initial temp., to 250°C at 25°C/min and to 280°C at 5°C/min.

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