un précipité qui est lavé par 2 fois 2 l. de $CHCl_3$. La fraction soluble dans $CHCl_3$ est concentrée à 200 ml. Par addition de 2 l. d'Et_2O, se forme un abondant précipité (A). Poids du précipité (A) obtenu: 20 g; rendement: 2%.

Etude préliminaire de (A). CCM: Si gel G Merck (Réf. 5553), solvant $CHCl_3$–MeOH (4:1). Mise en évidence de nombreuses taches dont une majoritaire R_f 0.44. Ces taches sont révélées par les réactifs suivants: réactif à l'hydroxylamine [7], rouge brique; réactif à la dinitrophénylhydrazine [7], bleu après 1 hr à 20°, orange verdâtre après 5 min à 105°. La tache majoritaire, isolée par chromatographie préparative sur plaque, fournit la réaction des esters [8]. Une hydroxylaminolyse menée sur cet ester permet la mise en évidence [9] des dérivés hydroxamiques d'un acide valérique et de l'acide acétique. L'hydrolyse acide de l'ester majoritaire donne le viburtinal.

Obtention du viburtinal. 7 g de précipité (A) sont dissous dans 100 ml de MeOH contenant 10% HCl. La solution est portée à ébullition sous réfrigérant à reflux pendant 1 hr. La solution d'hydrolyse, diluée par 300 ml d'eau est épuisée par 4 fois 200 ml de CCl_4. La phase organique, filtrée sur Na_2SO_4 anhydre est concentrée à 10 ml, et chromatographiée sur colonne de silice Merck (70–230 mesh. réf. 7734), diamètre 30 mm, hauteur 30 cm. L'élution est faite par des fractions de 500 ml du gradient suivant: pétrole; pétrole–EtOAc (19:1); pétrole–EtOAc (9:1). Le mélange pétrole–EtOAc (9:1) élue le viburtinal à l'état pur. Après évaporation du solvant, le viburtinal solubilisé dans l'eau chaude (60°) cristallise par refroidissement. Poids obtenu: 30 mg; rendement: 0.4%. Comportement chromatographique sur Si gel Merck G (réf. 5553): toluène–HCO_2Et–HCO_2H (5:4:1) R_f 0.61; C_6H_6–Et_2O–MeOH (17:2:1) R_f 0.77; CCl_4–EtOAc (7:3) R_f 0.73. (Analyse élémentaire: calculé C 74.99% H 5.03%, trouvé C 74.68% H 5.21%).

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IDENTIFICATION OF GIBBERELLINS A_{17}, A_{25}, A_{45}, ABSCISIC ACID, PHASEIC ACID, AND DIHYDROPHASEIC ACID IN SEEDS OF *PYRUS COMMUNIS*

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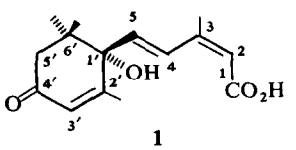
Key Word Index—*Pyrus communis*; Rosaceae; pear; gibberellins; abscisic acid; phaseic acid; dihydrophaseic acid; GC–MS.

Abstract—Gibberellin A_{17}, abscisic acid, and 4′-dihydrophaseic acid were identified by GC–MS of derivatized extracts from both immature and mature seeds of pear. Immature seeds also contained phaseic acid, gibberellins A_{25} and A_{45}, and two presumed mono-hydroxylated derivatives of GA_{45}, one of which was tentatively identified as 3β-hydroxy-GA_{45}. Several presumed metabolites of abscisic acid were detected in both mature and immature seeds.

Although ABA* (**1**) and gibberellins A_4, A_7 and A_9 have been identified [1–3] in extracts of apple seeds, pear seeds have received little attention until recently. Gil *et al.* [4] reported ABA-like, auxin-like, and GA-like activities in immature pear seeds, but none of the compounds was identified. In a preliminary publication [5] we reported the identification of GA_{17}, GA_{25}, GA_{45}, ABA and DPA (**3**) in immature seeds. In this paper we provide further information on these and other related compounds in both mature and immature seeds.



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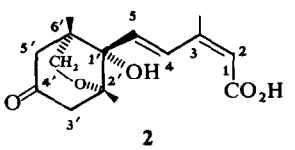

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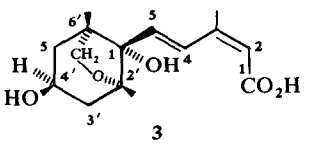
* Abbreviations used: ABA = (+)-abscisic acid; GA_x = gibberellin A_x: PA = phaseic acid; DPA = 4′-dihydrophaseic acid; MeTMS = methyl ester trimethylsilyl ether.



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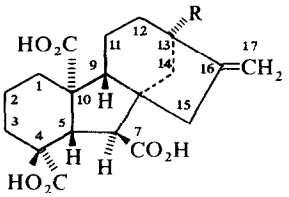
MeOH extracts, from both immature and mature pear seeds, were fractionated to yield acidic EtOAc (immature) or acidic *n*-BuOH (mature) fractions. The former were treated with PVP prior to derivatization and GC–MS, while the latter were subjected to TLC. In both mature and immature seeds the following compounds were identified by comparison of the MS of the derivatives, shown in parentheses, with reference spectra: ABA (**1**) (Me ester), GA_{17} (**5**) (MeTMS) and DPA (**3**) (MeTMS). Minor quantities of a compound, detected as the MeTMS derivative, had R_t slightly longer than, and an MS almost identical to, the MeTMS of DPA (**3**); this compound is thought to be *t,t*-DPA. Samples, prepared from immature

seeds, also contained PA (**2**) (Me ester), GA_{25} (**4**) (Me ester) and an unidentified isomer of GA_{25} (Me ester), GA_{45} (**6**) (MeTMS) and two compounds with M^+ 506 (MeTMS).

GA_{45} (**6**) was identified by comparison of the MS (Table 1) of the MeTMS derivative with that of a product [7] of the metabolism of desacetylxylopic acid (**8**) [8] by cultures of the mutant B1-41a of *Gibberella fujikuroi*. The MS of the two MeTMS derivatives with M^+ 506 showed fragmentation patterns analogous to GA_{45} MeTMS. In particular both contained intense *m/e* 156 ions, characteristic of 15-hydroxylated GAs. In addition one of them showed a prominent ion at *m/e* 129, characteristic of the



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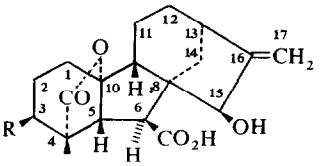

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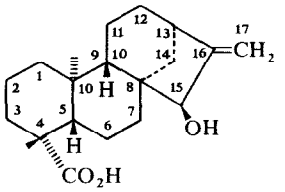

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Table 1. MS of $MeGA_{45}TMS$ (**6**)

418 (M^+, 100), 403 ($M^+ - 15$; 19), 358 (24), 328 ($M^+ - 90$; 5), 284 (15), 269 (13), 225 (18), 223 (12), 207 (17), 156 (90), 129 (11), 89 (11), 75 (51), 73 (99).

Table 2. MS of presumed Me 3β-hydroxy $GA_{45}TMS$

506 (M^+, 94), 491 ($M^+ - 15$; 38), 446 (23), 416 ($M^+ - 90$; 11), 377 (9), 287 (38), 282 (23), 223 (15), 207 (13), 156 (48), 143 (13), 129 (23), 89 (11), 75 (77), 73 (100).

Table 3. MS of Me-DPA-TMSi (**5**)

368 (M^+; 1), 353 ($M^+ - 15$; 2), 282 (3), 278 ($M^+ - 90$; 4), 246 (6), 220 (14), 199 (10), 188 (24), 159 (76), 159 (76), 125 (44), 122 (50), 121 (56), 177 (46), 109 (45), 75 (41), 73 (91), 43 (100).

Table 4. MS of presumed hydroxy-PA or keto-DPA

382 (M^+; 1), 367 ($M^+ - 15$; 3), 351 ($M^+ - 31$; 2), 268 (26), 228 (7), 196 (6), 159 (100), 125 (21), 117 (33), 75 (27), 73 (62), 69 (14), 43 (84).

Table 5. MS of presumed hydroxy-DPA

456 (M^+; 1), 441 ($M^+ - 15$; 1), 366 ($M^+ - 90$; 3), 307 (5), 280 (11), 276 (10), 219 (10), 194 (19), 159 (69), 125 (46), 117 (24), 75 (50), 73 (100), 43 (60).

TMS ether of a 1- or 3-hydroxy-GA, and was identical to the MS of a second metabolite obtained by culturing desacetylxylopic acid (**8**) with *G. fujikuroi*, mutant B1–41a. From these data structure (**7**) is indicated for one of these compounds. The other new compound is probably an x, 15-dihydroxy GA_9.

Both mature and immature seeds contained two additional compounds detected as the MeTMS derivatives with M^+ 382 and 456 and with MS fragmentation patterns similar to that of the Me ester of DPA (**5**) (Tables 3–5). These compounds are possibly derivatives of PA (**3**), one corresponding to a hydroxy-PA or a keto-DPA and the other to a hydroxy-DPA. Additional compounds with very similar spectra were observed in some samples. These may be epimers of these compounds, *trans*, *trans* isomers, or isomers differing in the position of hydroxyl groups.

EXPERIMENTAL

Plant material. 'Bartlett' pear fruits were collected from commercial orchards in the Sacramento River delta of California 85 days after full bloom. The seeds were removed from the flesh, frozen on dry ice, and lyophilized. Mature seeds of 'Bartlett' were purchased from a commercial source in Washington State, selected for uniformity, allowed to imbibe distilled H_2O for 24 hr, and stratified on moist filter paper at 4° for 4 wk.

Extraction of tissues and fractionation of extracts. The tissues (10 to 20 g) were ground in MeOH (10 ml per g), and the extracts were filtered through paper. H_2O was added to extracts of immature seeds to give a final concn of 80% MeOH. The extracts were concentrated *in vacuo* at 40° to the water phase, which was frozen, thawed, and centrifuged at 10000 rpm for 30 min. H_2O was added to the supernatant to give a total vol. of 20 ml, and the pH was adjusted to 8.0 with aqueous KOH. The extracts were first partitioned against PE (3 × 10 ml), then against EtOAc (3 × 10 ml). The pH of the aqueous phase was subsequently adjusted to 3.0 with HCl, and the extract partitioned against EtOAc (immature seed, 3 × 15 ml) or n-BuOH (mature seed, 3 × 15 ml). The bulked organic phase was washed once with distilled H_2O (10 ml), then evaporated to dryness *in vacuo* at 40°. The residue from the acidic EtOAc fraction of the extract of immature seeds was dissolved in KH_2PO_4 buffer (2 ml 0.1 M, pH 8.0), and shaken intermittently for 1 hr with PVP (50 mg per g dry wt original extract). The buffer was partitioned against PE (3 × 5 ml), the H_2O phase was adjusted to pH 3.0 with HCl and washed with EtOAc (3 × 10 ml), and the residue from the EtOAc was derivatized for GC without further treatment. The residue from the acidic *n*-BuOH fraction of extracts of mature seeds was chromatographed on pre-coated thin-layer plates (aluminium-backed Kieselgel 60, 200 × 200 × 0.25 mm) in EtOAc–$CHCl_3$–HOAc (15:5:1). After developing to a height of 10 cm, the zone from R_f 0.2 to 0.8 was eluted with H_2O-saturated EtOAc.

GC–MS. Residues from extracts of both immature and mature seeds were dissolved in a few drops of MeOH and methylated with CH_2N_2. A portion of the methylated extract was dissolved in Py and treated with Me_3SiCl and $(Me_3Si)_2NH$. Aliquots were examined by GC–MS, using a Pye 104 GLC coupled through a silicone membrane separator to an A.E.I. MS 30 dual beam mass spectrometer. Silanized glass columns (213.5 × 0.2 cm) were packed with 2% SE-33 on 80–100 mesh GasChrom Q. The He-flow rate was 25 ml/min., and column temp. was programmed from 180° to 280° at 3°/min. after an initial 4 min. at 180°. The MS were determined at 24 eV at a source temp. of 210° and a separator temp. of 190° with a scan speed of 6.5 sec per mass decade. The spectra were recorded by a DEC Linc-8 computer.

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TRITERPENOIDS FROM TWO HONG KONG EUPHORBIACEAE SPECIES*

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Key Word Index—*Claoxylon polot*; Euphorbiaceae; friedelin; 3β-hydroxy-30-nor-lupan-20-one and its acetate; betulinic acid; sitosterol; *Fluggea virosa*; Euphorbiaceae; friedelin; friedelan-3α-ol; friedelan-3β-ol; lupeol; glochidonol; glochilocudiol; betulonic acid and sitosterol.

The petrol extracts of both the leaves and stems of *Claoxylon polot* were examined separately by column chromatography on alumina. The former yielded friedelin and sitosterol, while the latter gave 3β-acetoxy-30-nor-lupan-20-one, sitosterol and 3β-hydroxy-30-nor-lupan 20-one, according to the order of elution from the column. 3β-Acetoxy-30-nor-lupan-20-one has not previously been isolated as a natural product, and its corresponding alcohol has only been isolated twice, first from *Ricihus communi* (Euphorbiaceae) [1], and later from *Carlina corymbosa* (Compositae) [2]. The ethanol extracts were then examined for acidic triterpenoids, only betulinic acid was isolated from that of the leaves.

Fluggea virosa (snowberry) was also analysed for its triterpene content. *F. microcarpa* previously yielded hexacosane, friedelin, friedelan-3α-ol and sitosterol from the trunk bark [3] and bergenin from the leaves [4].

Both the leaves and stems of *Fluggea virosa* were examined as for *Claoxylon polot*. The petrol extracts of the leaves gave in succession friedelin, friedelan-3α-ol and sitosterol, while that of the stems yielded friedelin, friedelan-3β-ol, lupeol, sitosterol, glochidonol, glochilocudiol [lup-20(29)-ene-1α,3β-diol]. Only betulonic acid was isolated from the ethanol extract of the stems. Glochilocudiol has only been isolated once from *Glochidion multiloculare* (Euphorbiaceae) [5].

EXPERIMENTAL

IR spectra were recorded for KBr discs; NMR spectra in $CDCl_3$ at 60 MHz using TMS as internal standard; optical rotations in $CHCl_3$ soln. Petrol had bp 60–80°. Known compounds were identified by TLC, mmp, IR and MS spectral comparisons with authentic samples.

Claoxylon polot (*Burm. f.*) *leaves.* Milled air-dried leaves (5 kg) were extracted 2 × with petrol for ten days. The combined extracts were concd and chromatographed on alumina (1.5 kg). Elution with petrol gave friedelin (0.03 g), mp 262–264°, IR ν_{max} cm^{-1}: 1720 (>O=O): with petrol–C_6H_6, sitosterol (0.7 g), mp 139–140°, IR ν_{max} cm^{-1}: 3300 (OH). The residue after extraction with petrol was extracted 2 × at room temp. with EtOH. The acidic solid (5 g) isolated through the Na salt, was treated with CH_2N_2 in Et_2O, and the product was chromatographed on alumina (100 g). Elution with petrol–C_6H_6 (1:1) gave prisms of methyl betulinate (0.05 g), mp 229–230°, IR ν_{max} cm^{-1}: 3550 (OH), 1720, 1174 (COOMe), 3080, 1650, 880 (>C=CH_2).

Stems. The petrol extract from the stems (10 kg) was chromatographed on alumina (1 kg). Elution with petrol yielded plates (0.03 g), mp 262–263° (from $CHCl_3$), $[\alpha]_D$ + 9.0° (Found: M^+-470. Calc for $C_{31}H_{50}O_3$: M^+-470), IR ν_{max} cm^{-1}: 1735, 1250 (OAc), 1695 (>C=O), NMR: δ 2.17 (3H, s, C<u>H</u>$_3$CO), 2.04 (3H, s, C<u>H</u>$_3$OCO), identical with a sample of 3β-acetoxy-30-nor-lupan-20-one prepared by ozonolysis of lupenyl acetate [6]. Elution with petrol–C_6H_6 yielded sitosterol (1.2 g); with C_6H_6, needles (0.02 g), mp 239–240°, $[\alpha]_D$ − 14.8°, MS: *m/e* 428 (M^+), IR ν_{max} cm^{-1}: 3470 (OH), 1695 (>C=O), identical with a sample of 3β-hydroxy-30-nor-lupan-20-one obtained by hydrolysis of 3β-acetoxy-30-nor-lupan-20-one [6]. The EtOH extract was treated as stated for the leaves, no acidic triterpenoids could be isolated.

Fluggea virosa (*Willd*) *Baill. leaves.* The petrol extract from the leaves (0.5 kg) was chromatographed on alumina (1 kg). Elution with petrol gave friedelin (0.7 g), with petrol–C_6H_6 yielded friedelan-3α-ol (0.01 g), mp 293–297°, IR ν_{max} cm^{-1}: 3600 (OH), and sitosterol (0.8 g). No acidic triterpenoid was isolated from the EtOH extract. *Stems.* The petrol extract from the stems (10 kg) was chromatographed on alumina (700 g).

* Part 15 in the series 'An Examination of the Euphorbiaceae of Hong Kong.' For Part 14, see Hui, W. H. and Li, M. M. (1977) *Phytochemistry* **16**, 113.