Grandisoic Acid, a Male-Produced Aggregation Pheromone from the Plum Curculio, *Conotrachelus nenuphar*

F. J. Eller* and R. J. Bartelt

Bioactive Constituents Research Unit, National Center for Agricultural Utilization Research, USDA/ARS/MWA, 1815 North University Street, Peoria, Illinois 61604

Received October 10, 1995[⊗]

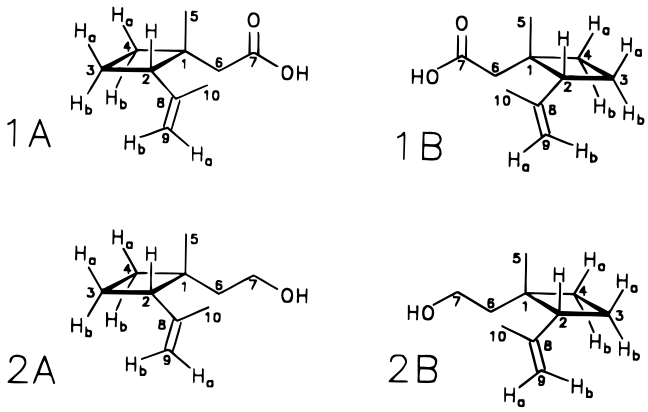
(+)-(1*R*,2*S*)-1-Methyl-2-(1-methylethenyl)cyclobutaneacetic acid (**1A**) was isolated from male plum curculios, *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae), feeding on plums and apples. Its structure was determined using achiral and chiral GLC, EIMS, optical rotation, and ^{1}H and ^{13}C NMR and was confirmed by synthesis. The racemic acid (a synthetic mixture of **1A** and its enantiomer) attracted both female and male plum curculios when used to bait traps placed in several species of fruit trees. It is suggested that **1A** is the major component of an aggregation pheromone of the plum curculio, and the trivial name, grandisoic acid, is proposed. Potential uses for grandisoic acid as well as possible ways to increase its effectiveness are discussed.

The plum curculio, *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae), is a major pest of stone and pome fruits east of the Rocky Mountains. It is responsible for decreased yields and lowered fruit quality.[1] The plum curculio is the only key apple pest for which there is no accurate, reliable, practical means for detecting its presence or estimating its density.[2,3] While male-produced pheromones have been identified for other economically important weevils in the family Curculionidae[4] and have become useful for monitoring populations,[5] no pheromone was previously known for the plum curculio. This research investigated sex-specific chemicals from plum curculios with the goal of identifying a pheromone that could be used to monitor plum curculio populations and improve the management of this serious pest.

Comparisons of the GLC chromatograms of male and female volatile collections revealed only one sex-specific peak in males. The peak shape (i.e., fronting) associated with this compound was reminiscent of that seen for geranic acid isolated from male pepper weevils[6] and suggested that the compound may be a carboxylic acid. This was further supported because the male-specific compound could be removed from the hexane extract using 5% Na_2CO_3 and the original compound could be regenerated by acidifying the Na_2CO_3 extract with 10% HCl and partitioning into hexane.

The mass spectral peaks at *m*/*z* 68 and 108 suggested a cyclobutane ring, as occurs in (+)-grandisol (**2A**), the major component of the pheromone of the boll weevil, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae).[7,8] The ion at 168 was consistent with a molecular formula of $C_{10}H_{16}O_2$, and the male-specific compound was tentatively identified as **1A**, the carboxylic acid analog of **2A**. Although racemic **1** (**1A** plus **1B**) and **1B** by itself were previously reported as synthetic intermediates,[9,10] neither **1A** nor **1B** have been previously reported as natural products.

Racemic **1** was synthesized as a standard for comparison. The male-derived compound and racemic **1** gave identical retention indices on two achiral GLC columns (1269 and 1290 on DB-1 and DB-5, respectively). The male-derived compound and racemic **1** also had identical mass spectra and ^{1}H and ^{13}C NMR spectra, and these were consistent with previous data.[9,11] The chemical shift data for racemic **1** were very similar to those reported for racemic grandisol (**2**),[12] with the exception of C-7 because of the different functionality.



The male-derived compound was reduced to the corresponding alcohol with $LiAlH_4$, and the retention indices were identical with those of racemic grandisol (**2**) on the achiral columns (1196 and 1219 on DB-1 and DB-5, respectively). The mass spectra of the reduced male-derived compound and racemic grandisol (**2**) were identical as well.

A chiral GLC column (CDX-B) did not separate **1A** from **1B**, but it did resolve racemic grandisol (**2**) into two peaks with retention indices of 1406 and 1412, respectively. After reduction to the alcohol, the male-specific compound (from both the southern and northern strains of plum curculios) produced only a single peak, with a retention index of 1406. Thus, both strains produce just one enantiomer, and this was identical in retention index to (+)-grandisol (**2A**) (derived from male boll weevils).[7,8] The retention index of synthetic (−)-grandisol (**2B**) was confirmed to be 1412.

Further confirmation of the stereochemistry was that the male-derived compound was found to have an optical rotation of $[\alpha]_D = +47.9$ (*c* 0.002 67, *n*-hexane), while Mori et al.[10] reported an optical rotation of $[\alpha]_D = -49.3$ (*c* 0.74, *n*-hexane) for (1*S*,2*R*)-1-methyl-2-(1-methylethe-

[⊗] Abstract published in *Advance ACS Abstracts,* April 1, 1996.

nyl)cyclobutaneacetic acid [**1B**]. All evidence thus supports the conclusion that the male-derived compound from plum curculios has the same ring stereochemistry as (+)-grandisol (**2A**) (i.e., 1*R*,2*S*), and thus the male-derived compound is (+)-(1*R*,2*S*)-1-methyl-2-(1-methylethenyl)cyclobutaneacetic acid (**1A**).

Traps baited with racemic **1** and attached to tree branches captured significantly ($t = 3.06$, $P = 0.012$, df = 10) more female plum curculios than did unbaited traps; numerical means ($n = 11$) were 3.45 and 1.27, respectively. Baited traps also captured significantly ($t = 2.79$, $P = 0.019$, df = 10) more male plum curculios than did unbaited traps; numerical means ($n = 11$) were 2.81 and 1.45, respectively. Therefore, racemic **1** has pheromonal activity, attracting both males and females. The name grandisoic acid is proposed for **1A** to reflect its structural relationship to grandisol[7] and grandisal.[8] The capture of some weevils by control traps is not surprising because plum curculios move within host trees principally by crawling[13] and reach fruit by walking rather than flying.[2]

The single-brooded northern strain and the double-brooded southern strain are reported to be reproductively incompatible,[14] but both strains were found to produce grandisoic acid. Therefore, it is unlikely that pheromone differences contribute to reproductive isolation of the strains.

There were obvious differences in the total number of plum curculios captured on the various tree species. The overall (baited plus unbaited, males plus females) mean number captured per trap for apple, plum, pear, and apricot were 4.0, 8.3, 1.5, and 1.5, respectively. The high number captured on plums is undoubtedly a reflection of its preference for this species.[15] These data suggest that sampling a plum tree within an apple orchard may be a more sensitive method to detect plum curculios than sampling apple trees in an orchard of apples only. In addition, because plum curculios are usually first found in trees near the edge of the planting,[1] traps placed there may detect weevil presence earlier than traps placed in the center of the orchard.

Currently, jarring (tapping branches over a cloth), is the most reliable adult monitoring technique for the plum curculio. However, jarring is generally not popular with growers because it can damage trees and cause some apples to fall.[16] The appearance of fresh egg-laying scars on developing fruit is a more convenient and less disruptive method for timing insecticide sprays;[17] however, such scars are only detectable several weeks after plum curculios arrive in orchards, which may be too late to achieve optimal control.[2] The identification of grandisoic acid may give pest managers a survey tool to replace jarring or searching for egg-laying scars. Such a monitoring tool could improve the integrated management of orchards and reduce pesticide usage on apples, peaches, plums, and cherries.

It may be possible to increase the sensitivity of traps baited with grandisoic acid in several ways. The presence of the antipode of (+)-grandisoic acid in the racemic mixture may render the mixture less active; this phenomenon has been shown for several insect species.[18,19] It is possible to separate the enantiomers of grandisoic acid from a racemic mixture by using quinine salts.[10] Host compounds may synergize the activity of the pheromone, as has been shown for other curculionid weevils, including boll weevils,[20−22] pine weevils,[23] pea leaf weevils,[24] and palm weevils.[25] Although a pheromone attractant apparently plays a role in mate-finding for the plum curculio, stridulation plays a role as well.[26] The males of other curculionid weevils have been demonstrated to both stridulate[27,28] and produce an aggregation pheromone.[23,29] It may therefore be possible to increase trap captures by combining sound with chemical attractants. Physical changes could be made to the trap design to increase its efficiency at attracting and retaining plum curculios. The use of food material (with or without an added toxin) within the trap may increase retention as well. It is hoped that the identification of grandisoic acid will stimulate further research on the development of an effective monitoring system for the plum curculio.

Experimental Section

General Analytical Procedures. Gas chromatography was performed using a Hewlett-Packard 5890 Series II GLC equipped with a flame ionization detector and a Hewlett-Packard 3396 Series II integrator. The columns used were a fused silica Durabond DB-5 (0.25-μm film thickness, 30 m × 0.25 mm i.d.), a fused silica Durabond DB-1 (1.0-μm film thickness, 15 m × 0.25 mm i.d.), and a fused silica Durabond CDX-B (chiral column, 0.25-μm film thickness, 30 m × 0.25 mm i.d.) (J & W Scientific, Folsom, CA). For all analyses, the temperature program was as follows: 50 °C initial temperature, 10 °C/min to 250 °C with helium as the carrier gas. The injector and detector temperatures were 220 and 250 °C, respectively. Injections of 1−2 μL were made in the splitless mode and changed to the split mode after 0.60 min. Retention indices were calculated relative to *n*-alkane standards.[30] Electron impact mass spectra (ca. 100 ng sample) were obtained using a Hewlett-Packard 5970 mass selective detector using an ionizing potential of 70 eV. Sample introduction was through a Hewlett-Packard 5890 GLC fitted with a DB-1 (0.25-μm film thickness, 15 m × 0.25 mm ID) capillary column. Proton and carbon nuclear magnetic resonance spectra (ca. 200 μg sample) were obtained using frequencies of 400 and 100 MHz, respectively, with a Bruker ARX 400 instrument with $CDCl_3$ as the solvent. Shifts are reported in parts per million (δ) relative to tetramethylsilane. Optical rotation (ca. 3 mg sample) was measured using a Perkin-Elmer Model 241 Polarimeter.

Insects. A laboratory culture of the southern non-diapausing strain of plum curculios was established from insects collected near Gainesville, FL. Insects were reared on thinning apples.[31] Insects representing the northern diapausing strain were collected in April through June near Peoria, IL. Cotton boll weevils were obtained from a laboratory culture maintained at the USDA Boll Weevil Research Lab (Starkville, MS).

Collection and Isolation. Volatiles were collected using Super-Q (Alltech Associates, Inc., Deerfield, IL) porous polymer filters and were extracted with hexane.[6] Volatiles were collected daily from individual unmated male and unmated female plum curculios feeding on apples or plums to detect sex-specific compounds. Volatile collections were pooled from males of the southern strain of plum curculios and the carboxylic acid isolated by extraction with 5% Na_2CO_3; this basic extract was

subsequently acidified with 10% HCl and extracted with *n*-hexane. Approximately 3 mg of grandisoic acid was isolated from 2000 male·day equivalents (southern strain) for an overall average of ca 1.5 μg per male·day. Similarly, volatiles were collected from males and females of the northern strain of plum curculios. Volatiles were also collected from unmated male cotton boll weevils feeding on cotton squares (var. DES-119) to provide a source of (+)-grandisol, (+)-(1*R*,2*S*)-1-methyl-2-(1-methylethenyl)cyclobutaneethanol (**2A**).

Reduction of Grandisoic Acid to Grandisol. The isolated grandisoic acid in hexane (ca. 10 μg in 1 mL) was treated with one drop of $LiAlH_4$ 1.0 M in diethyl ether) and neutralized with water and the hexane layer separated for analysis.

Racemic 1-Methyl-2-(1-methylethenyl)cyclobutaneacetic Acid (Grandisoic Acid) (1). Four grams of racemic 1-methyl-2-(1-methylethenyl)cyclobutaneethanol (grandisol I) (**2**) (Bedoukian, Inc.) was oxidized to the corresponding racemic aldehyde, 1-methyl-2-(1-methylethenyl)cyclobutaneacetaldehyde (grandisal) using pyridinium chlorochromate[32] (3.4 g, yield 86.1%). The racemic aldehyde was distilled under vacuum, and 3 g was subsequently oxidized to racemic **1** using $AgNO_3$ and NaOH:[33] nearly colorless oil (1.23g, yield 32.2%); eims *m*/*z* (rel int) 168 (1), 125 (12), 109 (14), 108 (35), 93 (9), 91 (3), 81 (6), 79 (11), 77 (10), 69 (11), 68 (100), 67 (86), 55 (11), 53 (30), 43 (35), 41 (41); ^{1}H NMR ($CDCl_3$) δ 2.61 (1H, m, H-2), 1.83 (1H, m, H-3a), 1.95 (1H, m, H-3b), 1.71 (1H, m, H-4a), 1.95 (1H, m, H-4b), 1.31 (3H, s, Me-5), 2.03 (1H, dd, J = 14.6 Hz, H-6a), 2.54 (1H, d, J = 14.6 Hz, H-6b), 4.85 (1H, m, H-9a), 4.65 (1H, m, H-9b), 1.64 (3H, s, Me-10); ^{13}C NMR ($CDCl_3$) δ 41.1 (C-1), 51.9 (C-2), 18.7 (C-3), 29.0 (C-4), 27.9 (C-5), 38.5 (C-6), 179.7 (C-7), 144.1 (C-8), 110.3 (C-9), 22.8 (C-10).

Field Bioassay. Synthetic racemic grandisoic acid (**1**) was tested for attractancy in a mixed species orchard near Peoria, IL, which had not been sprayed with pesticides for over 10 years. The experiment was set up as a paired test (baited vs unbaited traps, one trap per tree, with pairs of trees representing a single tree species and the trees not more than 10 m apart). There were 11 pairs of fruit trees in the experiment: five pairs of apples, three pairs of plums, two pairs of pears, and one pair of apricots. The treatments were randomly assigned to the trees of a pair. The grandisoic acid (**1**) was formulated as described for geranic acid,[6] and each lure contained ca. 5 mg. Commercial boll weevil traps (Great Lakes IPM, Vestaburg, MI) were used to sample for plum curculios. The lures were placed in the observation dome of the traps, and the traps were placed on the cut ends of branches which were as close to vertical as possible. The experiment was begun on May 6, 1994, traps were checked every 1−5 days, and the last weevil was captured on Sept 29, 1994. The captured insects were counted and sexed. The total number of males and females captured for the entire trapping period was analyzed by a paired t-test after log (x + 1) transformation using Statistix 4.1 Analytical Software (Tallahassee, FL).

Acknowledgment. Carroll Yonce (USDA/ARS, Byron, GA) supplied the southern strain of plum curculios, and David Weisleder (USDA/ARS, Peoria, IL) provided the nuclear magnetic resonance data. The boll weevils were obtained from Gerald McKibben (USDA/ARS, Starkville, MS), and Joseph Dickens (USDA/ARS, Mississippi State, MS) furnished the (−)-grandisol. Harold Tanner (Tanner's Orchard, Peoria, IL) donated the thinning apples, and Faye Halford kindly allowed the use of her orchard for the field test. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

References and Notes

(1) Chapman, P. J. *N.Y. St. Agric. Exp. Stn. Tech. Bull.* **1938**, 684.
(2) LeBlanc, J.-P. R.; Hill, S. B.; Paradis, R. O. *Environ. Entomol.* **1984**, *13*, 286−291.
(3) Whalon, M. E.; Croft, B. A. *Ann. Rev. Entomol.* **1984**, *29*, 435−470.
(4) Mayer, M. S.; McLaughlin, J. R. *Handbook of Insect Pheromones and Sex Attractants*; CRC Press: Boca Raton, FL, 1985.
(5) Cross, W. H. *Annu. Rev. Entomol.* **1973**, *18*, 17−46.
(6) Eller, F. J.; Bartelt, R. J.; Shasha, B. S.; Schuster, D. J.; Riley, D. G.; Stansly, P. A.; Mueller, T. F.; Shuler, K. D.; Johnson, B.; Davis., J. H.; Sutherland, C. A. *J. Chem. Ecol.* **1994**, *20*, 1537−1555.
(7) Tumlinson, J. H.; Hardee, D. D.; Geuldner, R. C.; Thompson, A. C.; Hedin, P. A.; Minyard, J. P. *Science* **1969**, *166*, 1010−1012.
(8) Hobbs, P. D.; Magnus, P. D *J. Am. Chem. Soc., Chem. Commun.* **1974**, 856−857.
(9) Ayer, W. A.; Browne, L. M. *Can. J. Chem.* **1974**, *52*, 1352−1360.
(10) Mori, K.; Tamada, S.; Hedin, P. A. *Naturwissenschaften* **1978**, *65*, 653−654.
(11) Zurfluh, R.; Dunham, L. L.; Spain, V. L.; Siddall, J. B. *J. Am. Chem. Soc.* **1970**, *92*, 425−427.
(12) Mori, K.; Miyake, M. *Tetrahedron* **1978**, *43*, 2229−2239.
(13) Owens, E. D.; Hauschild, K. I.; Hubbell, G. L.; Prokopy, R. J. *Ann. Entomol. Soc. Am.* **1982,** *75*, 357−362.
(14) Padula, A. L.; Smith, E. H. *Ann. Entomol. Soc. Am.* **1971,** *64*, 665−668.
(15) Quaintance, L.; Jenne, E. L. *U.S. Dept. Agric. Bur. Entomol. Bull.* **1912**, 103.
(16) LaFleur, G.; Hill, S. B. *J. Econ. Entomol.* **1987,** *80*, 1173−1187.
(17) Prokopy, R. J.; Coli, W. M.; Hislop, R. G.; Hauschild, K. I. *J. Econ. Entomol.* **1980**, *73*, 529−535.
(18) Vite, J. P.; Klimetzek, D.; Loskant, G. *Naturwissenschaften* **1976**, *63*, 582−583.
(19) Light, D. M.; Birch, M. C. *Naturwissenschaften* **1979**, *66*, 159−160.
(20) Hardee, D. D.; Wilson, N. M.; Mitchell, E. B.; Huddleson, P. M. *J. Econ. Entomol.* **1971**, *64*, 1454−1456.
(21) Dickens, J. C. *J. Chem. Ecol.* **1986**, *12*, 91−98.
(22) Dickens, J. C. *Entomol. Exp. Appl.* **1989,** *52*, 191−203.
(23) Booth, D. C.; Phillips, T. C.; Claesson, A.; Silverstein, R. M.; Lanier, G. N.; West, J. R. *J. Chem. Ecol.* **1983**, *9*, 1−12.
(24) Blight, M. M.; Wadhams, L. J. *J. Chem. Ecol.* **1987,** *13*, 733−739.
(25) Oehlschlager, A. C.; Pierce Jr., H. D.; Morgan, B.; Wilmalaratine, P. D. C.; Slessor, K. N.; King, G. G. S.; Gries, G.; Gries, R.; Borden, J. H.; Jiron, L. F.; Chinchilla, C. M.; Mexzan, R. G. *Naturwissenschaften* **1992**, *79*, 134−135.
(26) Mampe, C. D.; Neunzig, H. H. *Ann. Entomol. Soc. Am.* **1966**, *59*, 614−615.
(27) Hyder, D. E.; Oseto, C. Y. *J. Morphol.* **1989**, *201*, 69−84.
(28) Harman, D. M.; Kransler, G. A. *Ann. Entomol. Soc. Am.* **1969,** *62*, 134−136.
(29) Roseland, G. R.; Bates, M. B.; Oseto, C. Y. *Environ. Entomol.* **1990**, *19*, 1675−1680.
(30) Poole, C. F.; Schuette, S. A. *Contemporary Practice of Chromatography*; Elsevier: Amsterdam, 1984.
(31) Amis, A. A.; Snow, J. W. In *Handbook of Insect Rearing*; Singh, P., Moore, R. F., Ed.; Elsevier: Amsterdam, the Netherlands, 1985; Vol. I, pp 227−235.
(32) Webster, F. X.; Zeng, X.-N.; Silverstein, R. M. *J. Chem Ecol.* **1987**, *13*, 1725−1738.
(33) Pickett, J. A.; Williams, I. H.; Martin, A. P.; Smith, M. C. *J. Chem. Ecol.* **1980,** *6*, 425−433.

NP960102W