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ISOLATION OF 8-HYDROXYGERANIOL-8-*O*-β-D-GLUCOSIDE, A PROBABLE INTERMEDIATE IN BIOSYNTHESIS OF IRIDOID MONOTERPENES, FROM DEFENSIVE SECRETIONS OF *Plagiodera versicolora* AND *Gastrophysa viridula* (COLEOPTERA: CHRYSOMELIDAE)

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Abstract—8-Hydroxygeraniol and its 8-*O*-β-D-glucoside have been found as trace components in the defensive secretions of *Plagiodera versicolora* and *Gastrophysa viridula* larvae. This discovery supports the hypothesis that the evolution of the utilization of plant precursors by some chrysomelid species was favored by the plesiomorphic occurrence of a β-glucosidase and an oxidase in the defensive secretion of iridoid-producing species.

Key Words—Coleoptera, Chrysomelidae, defensive secretion, *Plagiodera versicolora*, *Gastrophysa viridula*, β-glucosidase, oxidase, 8-hydroxygeraniol, 8-hydroxygeraniol-8-*O*-β-D-glucoside.

INTRODUCTION

The larvae of leaf beetles belonging to the subtribe Chrysomelina and to the genus *Phratora* (Phyllodectina) secrete volatile compounds acting as irritants and quite active in repelling small predators (e.g., ants) (Pasteels et al., 1988). Although *Phratora* spp. are currently classified in a distinct subtribe (Seeno and Wilcox, 1982), their defensive glands are clearly homologous to those of the Chrysomelina, suggesting a monophyletic origin for all those taxa possessing

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these defensive glands (Pasteels and Rowell-Rahier, 1989; Pasteels, 1992). Recently published phylogenies based on the similarity of 12S and 16S mtDNA support this view (Hsiao, 1994).

In this group, most taxa synthesize de novo iridoid (cyclopentanoid) monoterpenes but others derive aromatic compounds from plant glucosidic precursors, e.g., salicylaldehyde in some *Chrysomela* and *Phratora* species and juglone in *Gastrolina depressa* (ref. in Pasteels et al., 1988). Circumstantial evidence based on the distribution of larval de novo and host-derived compounds among leaf beetle taxa suggests that the synthesis of iridoid monoterpenes is the plesiomorphic condition, whereas the secretion of host-derived compounds are apomorphic conditions that independently evolved at least three times (Pasteels and Rowell-Rahier, 1991). This suggestion is supported by mtDNA evidence (Hsiao, 1994).

The transformation of plant glucosides into volatile irritants only requires two enzymes located within the secretion stored in the glandular reservoirs. It was suggested (Pasteels et al., 1990) that the repeated evolution of such utilization of plant glucosides was favored by the plesiomorphic occurrence of a β-glucosidase and an oxidase in the defensive secretions of species producing iridoid monoterpenes. It was similarly hypothesized that the two enzymes are required in the final steps of the biosynthesis of the latter compounds and, thus, that small changes in the specificity of these preexisting enzymes would allow the shift from de novo synthesis to the utilization of plant precursors in the insect defensive chemistry (Pasteels et al., 1990). This suggestion was supported by the presence of glucose in the secretion of species producing iridoid monoterpenes and by the demonstration of a β-glucosidase activity in the secretion of at least three species: *Plagiodera versicolora* (Duffey and Pasteels, unpublished results), *Phratora tibialis* and *Ph. laticollis* (Soetens et al., 1993). The above hypothesis postulates that a monoterpene glucoside should be one of the biosynthetic precursors of the defensive iridoid monoterpenes and that this glucoside should be present in the glandular reservoir in which the final steps leading to the iridoid monoterpenes occur.

The purpose of this work was to search in the defensive secretions of *Plagiodera versicolora* and *Gastrophysa viridula* for this hypothetical monoterpene glucoside and its aglycone, and thus, to refine the biosynthetic scheme recently proposed by Lorenz et al. (1993). The larvae of *P. versicolora* used in this study secrete two iridoid monoterpenes, plagiodial and plagiolactone, and those of *G. viridula*, two related compounds, chrysomelidial and epichrysomelidial (Pasteels et al., 1982). Using deuterated nor-precursors, Lorenz et al. (1993) demonstrated that these compounds are biosynthesized by the larvae, through a pathway similar to that established for the biosynthesis of iridoids in plants (Inouye and Uesato, 1987). Geraniol formed from mevalonic acid is ω-hydroxylated into 8-hydroxygeraniol, which is further oxidized into

8-oxocitral. The latter is finally cyclized to afford plagiodial or chrysomelidial, depending on the species.

METHODS AND MATERIALS

Biological Material

Plagiodera versicolora and *Gastrophysa viridula* were collected near Brussels on various *Salix* species and on *Rumex obtusifolia*, respectively. They were raised in the laboratory in plastic, 500-ml containers with perforated transparent plastic lids. The bottom of the containers were covered with moist paper and the insect fed with fresh leaves of *Salix babylonica* and *Salix fragilis* × *alba* (*P. versicolora*) or *Rumex obtusifolia* (*G. viridula*), renewed every two days.

Collection of Secretions. To increase the probability of finding the biosynthetic precursors, secretions of larvae that were active in the process of synthesis were collected. Since the secretion of a larvae is renewed in about 24 hr, third-instar larvae were milked twice a day and on each consecutive day until pupation. These secretions were collected in glass capillaries half filled with methanol in order to inactivate as soon as possible the enzymes present in the secretion. No attempt was made to quantify the amount of secretion collected, but the secretions of several hundreds of larvae were collected in this way and stored in methanol.

Isolation of 8-Hydroxygeraniol [2,6-dimethyl-2(E),6(E)-octadiene-1,8-diol] (1), and its 8-O-β-D-glucoside (2) from Defensive Secretion of P. versicolora *(Figure 1)*

The constituents of the defensive secretion of third-instar larvae of *P. versicolora* were partitioned between CH_3OH/H_2O/hexane (5 : 1 : 5), by stirring magnetically for 10 min. The phases were then allowed to separate and the

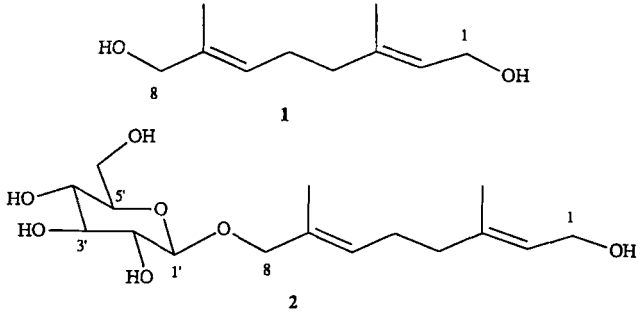

FIG. 1. Structures of the trace constituents found in the defensive secretions of *Plagiodera versicolora* and *Gastrophysa viridula*.

upper hexane layer removed with a Pasteur pipet. This procedure was repeated five times, after which most of the iridoid monoterpenes (plagiodial and plagiolactone) were concentrated in the hexane phase. The CH_3OH/H_2O phase was evaporated under reduced pressure, leaving 4.7 mg of material. A TLC analysis of the latter (SiO_2, $CHCl_3/CH_3OH$, 8:2, visualized by spraying with anisaldehyde or vanillin/conc. H_2SO_4) showed the presence of free glucose (major spot, $Rf = 0.1$), and a small amount of plagiodial ($Rf = 0.8$), accompanied by two faint spots ($Rf = 0.4$ and 0.7), exhibiting characteristic blue-green (anisaldehyde) or red colors (vanillin). This mixture was flash chromatographed on a silica gel column (5 mm diameter), eluted with $CHCl_3/CH_3OH$ (95:5, 90:10, and 70:30, 20 ml each). This separation afforded plagiodial and glucose, identified by direct comparison with authentic samples (GC or TLC, ^1H NMR), as well as two compounds corresponding to the aforementioned spots as $Rf = 0.7$ and 0.4. These were isolated in very small amounts and thus could not be weighed accurately. The analyses performed on these samples suggest that less than 50 μg of each compound was isolated.

8-Hydroxygeraniol (1) (Figure 1)

The compound at $Rf = 0.7$ was identified as 8-hydroxygeraniol by comparison with an authentic sample (Aldrich) in TLC ($CHCl_3/CH_3OH$, 95:5), by coinjection in GC on two different capillary columns (Rescom, 25 m, 0.32 mm diameter): OV-1701, 160°C isothermal and PEG 200°C isothermal, and by GC-MS (Finnigan, ITD 800, coupled to a Tracor gas chromatograph equipped with an OV-1701 column, isothermal at 180°C. CI-MS (isobutane): *m/z* 153 [10, $(M + H - H_2O)^+$]; 135 [100, $(M + H - 2H_2O)^+$]; 123 (10); 121 (15); 109 (12); 107 (40); 95 (38); 93 (25); 81 (22); 67(10).

8-Hydroxygeraniol-8-O-β-D-glucoside (2) (Figure 1)

The ^1H NMR spectrum (CD_3OD, 600 MHz, Varian Unity 600) of the compound at $Rf = 0.4$ showed that it was still accompanied by impurities. It was rechromatographied on a Pasteur pipet containing silica gel previously washed with redistilled $CHCl_3/CH_3OH$ (1:1) and subsequently heated at 140°C for 1 hr. The eluent was a 85:15 mixture of the same redistilled solvents. The purity of the sample thus obtained was now sufficient for spectroscopic analyses. CI-MS (NH_3): *m/z* 350 [100, $(M + NH_4)^+$]; 333 [58, $(M + H)^+$]; 332 (40, M^+); 315 [56, $(M + H - H_2O)^+$]; 304 (32); 256 (14); 212 (14); 180 (40); 169 (12); 162 (15); 153 (18); 135 (40); 118 (20); 110 (30); 108 (20). ^1H NMR (CD_3OD, 600 MHz, Varian Unity 600): 5.48 (H-2 or H-6, t, 7.0 Hz); 5.35 (H-6 or H-2, t, 7.0 Hz); 4.23 (H-1′, d, 8.0 Hz); 4.04 and 4.19 *H_2-8, AB system, $J_{AB} = 11.0$ Hz); 4.07 (H_2-1, d, 7.0 Hz); 3.85 (H-6′a, dd, 12.2, 2.5

Hz); 3.64 (H-6'b, dd, 12.2, 5.5 Hz); 3.20 (H-2', dd, 9.0, 8.0 Hz); 2.18 (H_2-5, m); 2.08 (H_2-4, t, 7.0 Hz); 1.68 and 1.66 (2 × 3H, s, H_3-9 and H_3-10).

Acetylation of a few micrograms of **2** overnight with the mixture Ac_2O-pyridine afforded pentaacetate **3** (Figure 2) that was purified by chromatography on a Pasteur pipet filled with silica gel, with tridistilled hexane/AcOEt, 7:3, as eluent. ^1H NMR spectrum of **3** [$CDCl_3$, 600 MHz, 48,448 scans: 5.35 (2H, m, H-2 + H-6); 5.18 (H-3', t, 9.0 Hz); 5.08 (H-4', t, 9.0 Hz); 5.0 (H-2', dd, 9.0, 7.8 Hz); 4.58 (H_2-1, d, 7.5 Hz); 4.47 (H-1', d, 7.8 Hz); 4.25 (H-6'a, dd, 12.0, 5.0 Hz); 4.14 (H-6'b, dd, 12.0, 2.0 Hz); 4.12 and 3.95 (2H, AB system, J_{AB} = 11.5 Hz); 2.3 (4H, m, H_2-4 + H_2-5); 2.08, 2.04, 2.02, 2.0, 1.98 (5 × CH_3COO, s); 1.68 and 1.64 (2 × 3 H, s, H_3-9 and H_3-10)].

Compounds **1** and **2** were also detected in the defensive secretion of *Gastrophysa viridula* larvae by TLC comparison with the secretion of *P. versicolora* (*Rf* and characteristic color with anisaldehyde/conc. H_2SO_4 spray).

Synthesis of 8-Hydroxygeraniol-8-O-β-D-glucoside (2) (Figure 3)

Koenigs-Knorr Reaction. See Reyle et al. (1950). Typically, α-bromotetraacetylglucose (0.375 g, 0.91 mmol) and 8-hydroxygeraniol (0.210 g, 1.24 mmol) were dissolved in anhydrous toluene. Ag_2CO_3 (0.15 g, 0.54 mmol), prepared according to the procedure of Organic Synthesis (1955) and dried for three days in a desiccator containing P_2O_5, and anhydrous $CaSO_4$ (0.180 g, 1.32 mmol) were added and the mixture was stirred at room temperature in the dark. The reaction was followed by TLC and was stopped after 30 hr by dilution with CH_2Cl_2 and filtration on Celite. The two isomeric tetraacetyl glucosides **4** and **6** (Figure 3) were only resolved in TLC with toluene/AcOEt mixtures. Flash chromatography (eluent: toluene/AcOEt 6:4) of the reaction mixture afforded 20 mg of **4**, 40 mg of **6**, and 117 mg of a mixture of the two compounds.

Compound **4.** ^1H NMR ($CDCl_3$, 250 MHz, Bruker WM 250): 5.39 (H-2 and H-6, m); 5.22 (H-4', dd, 9.5, 9.5 Hz); 5.08 (H-3', dd, 9.5, 9.5 Hz); 5.00 (H-2', dd, 9.5, 8.0 Hz); 4.49 (H-1', d, 8.0 Hz); 4.25 (H-6'a, dd, 12.0, 5.0 Hz); 4.15 (H_2-1, d, 7.0 Hz); 4.13 (H-6'b, dd, 12.0, 2.7 Hz); 4.16 and 3.96 (H_2-8, AB system, J_{AB} = 12.0 Hz); 3.65 (H-5', m); 2.15 and 2.10 (H_2-4 and

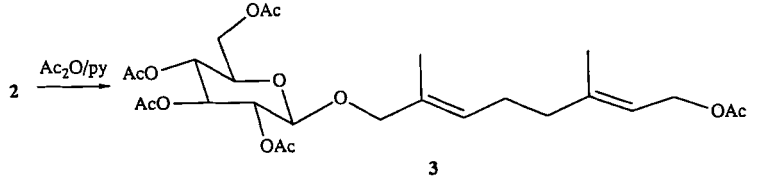

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FIG. 2. Synthesis of pentaacetate **3** from 8-hydroxygeraniol-8-*O*-β-D-glucoside **(2).**

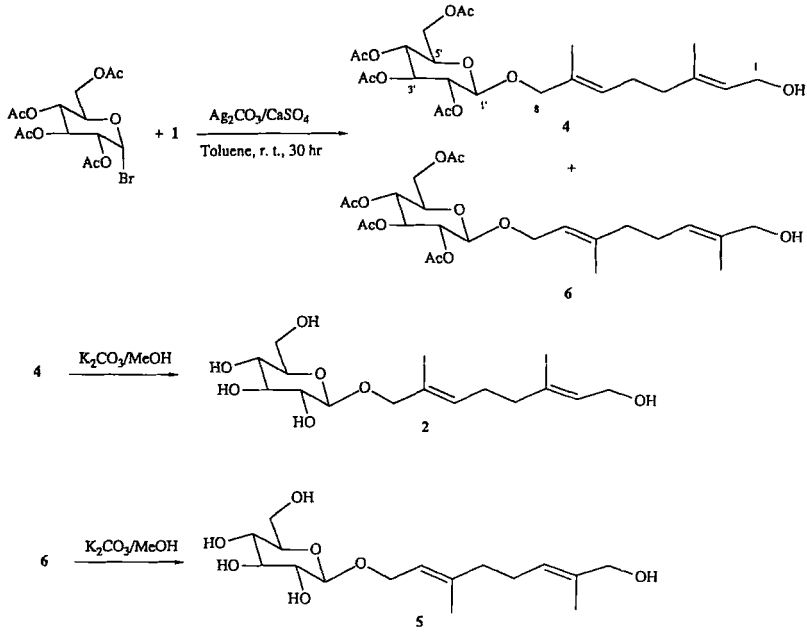

FIG. 3. Synthesis of 8-hydroxygeraniol-8-*O*-β-D-glucoside (**2**) and 8-hydroxygeraniol-1-*O*-β-D-glucoside (**5**).

H_2-5, m); 2.09, 2.03, 2.01, 2.0 (4 × CH_3COO, s); 1.68 and 1.60 (H_3-9 and H_3-10, s).

Compound **6.** ^1H NMR ($CDCl_3$, 250 MHz, Bruker WM 250): 5.38 (H-2 or H-6, t, 6.5 Hz); 5.26 (H-6 or H-2, t, 6.5 Hz); 5.22 (H-4′, dd, 9.5, 9.5 Hz); 5.07 (H-3′, dd, 9.5, 9.5 Hz); 4.98 (H-2′, dd, 9.5, 8.0 Hz); 4.54 (H-1′, d, 8.0 Hz); 4.23 (H-6′a, H-6′b and H_2-1, m); 4.01 (H_2-8, bd, 6.0 Hz); 3.65 (H-5′, m); 2.10 (H_2-4 and H_2-5, m); 2.08, 2.04, 2.02, 2.0 (4 × CH_3COO, s); 1.66 (H_3-9 and H_3-10, s).

Hydrolysis of ***4*** *and* ***6*** *(Figure 3).* Compounds **4** and **6** were each treated for 48 hr with a saturated methanolic solution of K_2CO_3. The CH_3OH was evaporated under vacuum, water was added, and the aqueous solution extracted successively with $CHCl_3$ and *n*-BuOH. The *n*-BuOH extract containing the glucoside was dried under reduced pressure with toluene and chromatographied on a small silica gel column (eluent: CH_2Cl_2/CH_3OH, 85:15). This procedure afforded 7 mg of **2** from 20 mg of **4** and 12 mg of the isomer **5** from 40 mg of **6.**

8-Hydroxygeraniol-8-O-β-D-glucoside ***(2).*** Amorphous; CI-MS and ^1H NMR spectrum identical to those of the natural compound (see above).

8-Hydroxygeraniol-1-O-β-D-glucoside (5). Amorphous; CI-MS identical to that of **2**; ^{1}H NMR (CD_3OD, 250 MHz, Bruker WM 250): 5.38 (H-2 and H-6, t, 6.5 Hz); 4.26 (H_2-1, AB of ABX, J_{AB} = 12.0 Hz, J_{AX} = J_{BX} = 6.5 Hz); 3.90 (H_2-8, s); 3.86 (H-6'a, dd, 12.0, 2.2 Hz); 3.66 (H-6'b, dd, 12.0, 5.3 Hz); 3.36 to 3.15 (H-2', H-3', H-4'); 2.17 and 2.11 (H_2-4 and H_2-5, m); 1.69 and 1.64 (H_3-9 and H_3-10, s).

RESULTS

Examination of the crude defensive secretion of larvae of *Plagiodera versicolora* by TLC ($CHCl_3/CH_3OH$, 8:2, anisaldehyde conc. H_2SO_4 spray) allowed us to detect the presence of the familiar monoterpenes, plagiodial and plagiolactone, together with a much more polar compound identified as glucose by comparison with an authentic sample (Pasteels et al., 1982). However, after partitioning this secretion between the two phases of a CH_3OH/H_2O/hexane (5:1:5) system, most of the plagiodial and plagiolactone were transferred into the hexane layer. TLC analysis of the concentrated CH_3OH/H_2O phase now allowed detection of, besides glucose (Rf = 0.1), two more spots at Rf = 0.4 and 0.7.

The compounds corresponding to these spots were isolated by careful chromatography on small silica gel columns. The compound at Rf = 0.7 was easily identified as 8-hydroxygeraniol **(1)** (Figure 1) by TLC, GC, and GC/MS comparison with an authentic sample. The spectral properties of the more polar compound (Rf = 0.4) (see Methods and Materials) suggest that it is 8-hydroxygeraniol-8-*O*-β-D-glucoside **(2)** (Figure 1). In particular, the CI-MS shows a $[M + H]^+$ ion at *m/z* 333, together with fragment ions at *mz* 180 and 162, characteristic for an hexose sugar, and at *m/z* 169, 153, and 135, characteristic for an acyclic dihydroxylated monoterpene. The ^{1}H NMR spectrum at 600 MHz was in complete agreement with the proposed structure. The glucose was located at C-8 of the 8-hydroxygeraniol moiety since the CH_2-8 appears as an AB system at δ 4.04 and 4.19 (3.95 and 4.12 in pentaacetate **3**) and CH_2-1 as a doublet at δ 4.07 (4.58 in **3**). This identification was further confirmed by comparison of **2** with synthetic 8-hydroxygeraniol-8-*O*-β-D-glucoside and 8-hydroxygeraniol-1-*O*-β-D-glucoside **(5)**, obtained by a Koenigs-Knorr reaction (Reyle et al., 1950) between α-bromotetraacetylglucose and 8-hydroxygeraniol, followed by separation of the two isomeric tetraacetyl glucosides **4** and **6**, and, finally, basic hydrolysis of the acetate groups (Figure 3). The identity of the natural and synthetic compounds also defines the absolute configuration of the glucose moiety as D.

The presence of 8-hydroxygeraniol and its 8-*O*-β-D-glucoside in the defensive secretion of *Gastrophysa viridula* was demonstrated by careful TLC comparisons with authentic material.

DISCUSSION

The presence in the defensive secretion of *Plagiodera versicolora* of traces of 8-hydroxygeraniol-8-*O*-β-D-glucoside **(2)**, and of both 8-hydroxygeraniol **(1)** (Figure 1) and free glucose, together with the demonstration of a β-glucosidase activity in this secretion (Duffey and Pasteels, unpublished results support the view that 8-hydroxygeraniol 8-*O*-β-D-glucoside **(2)** is the first or immediate precursor of the glandular chemistry. Thus, it seems likely that the glucoside **2** is rapidly hydrolyzed in the reservoir, liberating 8-hydroxygeraniol, which is further transformed according to the scheme put forward by Lorenz et al. (1993). The final steps of the biosynthesis occur in the gland reservoirs, outside the glandular cell, and require several enzymes, among which are a β-glucosidase and at least one oxidase. Since both 8-hydroxygeraniol 8-*O*-β-D-glucoside **(2)** and 8-hydroxygeraniol **(1)** (Figure 1) were also found in the defensive secretion of *Gastrophysa viridula* producing other iridoid monoterpenes—chrysomelidial and epichrysomelidial (Pasteels et al., 1982)—the first steps in the biosynthesis of iridoids in these chrysomelid larvae must be similar and only differ after the formation of 8-hydroxygeraniol and its subsequent oxidation into 8-oxocitral (see also Lorenz et al., 1993).

Our results thus support the hypothesis that the evolution of the utilization of plant precursors by some species was facilitated by preexisting enzymes in the glandular reservoir. In the absence of information on the biosynthesis of iridoid monoterpenes in leaf beetles larvae, it was suggested that the precursor of iridoid aldehydes could be a glucoside of an already cyclized monoterpene (Pasteels et al., 1990). This obviously was an incorrect guess, since we have now shown that the glucoside is formed earlier in the biosynthetic pathway. In this context, it is interesting to point out that the classical scheme of iridoid glucoside biosynthesis in plants postulates that the glucosidation of the monoterpene aglycone is always effected after the cyclization of the latter into a cyclopentane ring (reference in Inouye and Uesato, 1987). However, this view has been questioned recently, following the isolation of several acyclic monoterpene glucosides in iridoid glucoside-producing plants (Gross, 1985; Gross et al., 1987; Gering-Ward and Junior, 1989). Thus, the presence of glucosylated precursors at an early stage of iridoid biosynthesis could be a common feature in both plants and insects.

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REFERENCES

GERING-WARD, B., and JUNIOR, P. 1989. Neue Inhaltstoffe aus *Penstemon digitalis*. *Planta Med.* 55:75–78.

GROSS, G.A. 1985. Dissertation. ETH Zürich No. 7800.

GROSS, G.A., STICHER, O., and ANKLIN, C. 1987. Zwei weitere Iridoid-glykoside und ein Monoterpen-glykosid aus *Sambucus ebulus* L. (Caprifoliaceae). *Helv. Chim. Acta* 70:91–101.

HSIAO, T.H. 1994. Molecular techniques for studying systematics and phylogeny of Chrysomelidae, *in* P. Jolivet, E. Petitpierre, and M. Cox (eds.). Novel Aspects of Biology of Chrysomelidae, Kluwer, Dordrecht.

INOUYE, H., and UESATO, S. 1987. Biosynthesis of iridoids and secoiridoids. *Progr. Chem. Org. Nat. Prod.* 50:169–236.

LORENZ, M., BOLAND, W., and DETTNER, K. 1993. Biosynthesis of iridodials in the defense glands of beetle larvae (Chrysomelinae). *Angew. Chem. Int. Ed.* 32:912–914.

HORNING, E.C. (ed.). 1955. β-d-glucose-2,3,4,6-tetraacetate Organic Synthesis, pages 434–435, Collective Volume III. John Wiley, New York.

PASTEELS, J.M. 1992. The value of defensive compounds as taxonomic characters in the classification of leaf beetles, *Biochem. Syst. Ecol.* 21:135–142.

PASTEELS, J.M., and ROWELL-RAHIER, M. 1989. Defensive glands and secretions as taxonomical tools in Chrysomelidae. *Entomography* 6:423–432.

PASTEELS, J.M., and ROWELL-RAHIER, M. 1991. Proximate and ultimate causes for host plant influence on chemical defense of leaf beetles (Coleoptera: Chrysomelidae). *Entomol. Gen.* 15:227–235.

PASTEELS, J.M., BRAEKMAN, J.C., DALOZE, D., and OTTINGER, R. 1982. Chemical defence in chrysomelid larvae and adults. *Tetrahedron* 38:1891–1897.

PASTEELS, J.M., BRAEKMAN, J.C., and DALOZE, D. 1988. Chemical defense in the Chrysomelidae, pp. 233–252 *in* P. Jolivet, E. Petitpierre, and T. H. Hsiao (eds.). The Biology of Chrysomelidae. Kluwer, Dordrecht.

PASTEELS, J.M., DUFFEY, S., and ROWELL-RAHIER, M. 1990. Toxins in chrysomelid beetles. Possible evolutionary sequence from de novo synthesis to derivation from food-plant chemicals. *J. Chem. Ecol.* 16:211–222.

REYLE, K., MEYER, K., and REICHSTEIN, T. 1950. Partialsynthese von Convallotoxin. Glykoside und Aglykone, 61. Mitteilung. *Helv. Chim. Acta* 33:1541–1546.

SEENO, T.N., and WILCOX, J.A. 1982. Leaf beetle genera (Coleoptera: Chrysomelidae). *Entomography* 1:1–221.

SOETENS, P., PASTEELS, J.M., and DALOZE, D. 1993. A simple method for in vivo testing of glandular enzymatic activity on potential precursors of larval defensive compounds in *Phratora* species (Coleoptera: Chrysomelidae). *Experientia* 49:1024–1026.