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The Preparation and Bioactivities of (−)-Isovelleral[1,2]

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Abstract—The resolution of synthetic (±)-isovelleral (**1**), via chromatographic separation of the two diastereomers of the (−)-menthoxyacetic acid diester of the corresponding (±)-diol (**3**), yielded both enantiomers of the bioactive fungal metabolite (+)-isovelleral (**1**). While the antimicrobial and cytotoxic activities of the two enantiomers are comparable, natural (+)-**1** is approximately 10 times more mutagenic towards Ames' tester strain TA98 than (−)-**1**. The two enantiomers of the cyclopropane ring isomer **2** also possess negligible mutagenicity compared to (+)-**1**. Both (+)-**1** and (−)-**1** have the same affinity for the vanilloid receptor, but significant different affinity for the dopamine D1 receptor. © 1997 Elsevier Science Ltd.

Introduction

(+)-Isovelleral (**1**) is a fungal sesquiterpene containing an unsaturated dialdehyde functionality, formed enzymatically in the fruit bodies of, for instance, *Lactarius vellereus* as a response to injury.[3] It is a strong antibiotic and antifeedant, and apparently protects the fruit bodies against parasites and predators.[4] In addition, it possesses potent mutagenic activity in both bacteria (Ames' Salmonella assay)[5] and mammalian cells (V79/HGPRT assay).[6] As with other unsaturated dialdehydes, the biological activities of isovelleral (**1**) are known to depend on the presence of an intact unsaturated dialdehyde functionality, and it is generally recognized that there is a correlation between the reactivity with nucleophiles and, for example, the antibiotic activity of this class of compounds.

However, some bioactivities (e.g., the affinity for certain nerve cell receptors)[7] suggest that isovelleral (**1**) can also interact with constituents of biochemical systems in a more selective way, and the possibility that the stereochemistry, including the absolute stereochemistry, influences such activities is intriguing. A few investigations in which the bioactivities of enantiomers of unsaturated dialdehydes are compared have been performed. It was for example shown that both enantiomers of polygodial possess similar levels of antifeedant activity, phytotoxicity, fish toxicity, and taste to human tongue, but react at different rates with enantiomers of 1-phenylethylamine.[8,9] Notably, (−)-warburganal gave a stronger allergic contact dermatitis response than racemic (±)-warburganal on guinea pigs, which were sensitized to (−)-polygodial (having the same absolute configuration as (−)-warburganal).[10] Recently we reported that a racemic sample of isovelleral (**1**) possessed lower mutagenic activity compared to the natural product.[11] Hence, the aim of this study was to prepare (−)-isovelleral (**1**) and compare its bioactivities with those of (+)-**1**, (−)-**2**, (+)-**2**, and other unsaturated dialdehydes, with the hope that this comparison may lead to further insight into structure–activity relationships for this class of terpenoids.

Results

Two total syntheses of isovelleral (**1**) have been reported, one enantioselective leading to the natural (+)-enantiomer **1**,[12] and one yielding racemic isovelleral.[13,14] The unnatural (−)-enantiomer of **1** could therefore be prepared either by adopting the first synthesis to produce the (−)-enantiomer, or by the

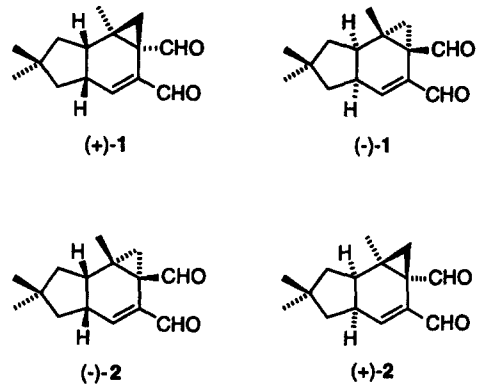

Figure 1. Structures of the isovelleraloids.

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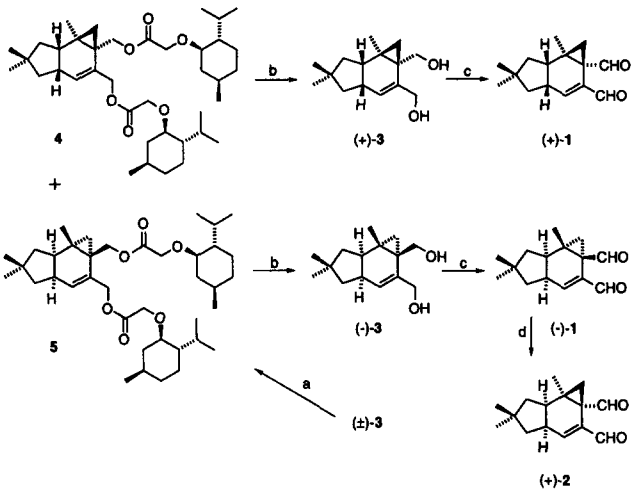

Scheme 1. (a) (−)-Menthoxyacetyl chloride, pyridine; (b) KOH, water; (c) $(COCl)_2$, DMSO, CH_2Cl_2, Et_3N; (d) 180 °C, toluene.

resolution of the racemic mixture obtained in the second. As we were interested in some of the intermediates formed in the racemic synthesis, we employed the latter to obtain the racemic diol (±)-**3**, which could be transferred to the two diastereomeric esters **4** and **5** by treatment with (−)-menthoxyacetyl chloride in pyridine (Scheme 1).[8] The diastereomers **4** and **5** were separated by chromatography and compared with the diester prepared from the diol of the natural (+)-isovelleral (**1**). The latter was identical in all respects to compound **4**, no traces of its diastereomer **5** could be detected by ^1H NMR spectroscopy and (+)-isovelleral (**1**) isolated from *Lactarius vellereus* is at least 99% enantiomerically pure. The saponification of **4** and **5** and oxidation of the resulting diols yielded (+)-**1** and (−)-**1**. The isomers (−)-**2** and (+)-**2** were prepared by thermal isomerisation[15] of (+)-**1** and (−)-**1**, respectively.

As discussed above, the unsaturated dialdehydes in general and isovelleral (**1**) in particular possess a number of different bioactivities.[5,7,8,16,17] Primarily we were interested in comparing the activities that can be considered to be caused by a general interference with the growth-related processes of the organism, due to their reactivity, with activities that are possibly caused by a specific influence on molecular targets such as receptors. The antimicrobial and cytotoxic activities of (+)-isovelleral (**1**) and its isomer (−)-**2** have been reported,[16] and although (+)-**1** is slightly more active than (−)-**2** the difference is small. In this investigation the antimicrobial activity of (+)-**1**, (−)-**1**, and (+)-**2** towards the bacteria *Bacillus brevis*, *B. subtilis*, *Enterobacter dissolvens*, and *Micrococcus luteus*, and the fungi *Paecilomyces variotii*, *Nematospora coryli*, and *Penicillium notatum*, as well as the cytotoxicity towards BHK 21, L1210, and B16-F1 cells, were compared. The inhibitory concentrations (IC_{50}) against microorganisms and mammalian cells are between 1 and 5 μg/mL, and similar for the three compounds. Also, the phytotoxic activity of the isovelleraloids are unaffected by the absolute as well as the relative stereochemistry of the compounds, all three cause approximately 50% inhibition of the germination of seeds of *Seteria italica* at 1 μg, and of *Lepidium sativum* at 2 μg. However, when assayed in the Ames' mutagenicity assay towards the *Salmonella typhimurium* strain TA98, the mutagenic response of (+)-**1** was 244 revertants per μg (0, 0.5, 1, and 2 μg/plate tested, plates triplicated, correlation coefficient 0.97) while that of (−)-**1** was only 23 revertants per μg (same procedure, correlation coefficient 0.95). (−)-**2** has previously been shown to possess only approximately 10% of the mutagenicity of (+)-**1**,[5] and is not significantly mutagenic towards mammalian cells,[6] and (+)-**2** was found to be completely devoid of mutagenic activity in this investigation. It appears as if (+)-isovelleral (**1**) has the ability to interact in a highly specific way with the genetic material, and the mutagenic potency of (+)-**1** towards mammalian cells compared to well-known mutagens ((+)-**1** induced 39×10^6 mutations at 0.4 μg/mL in Chinese hamster lung fibroblast cells while ethyl methanesulfonate induced 64×10^6 mutations at 200 μg/mL),[6] motivates further studies of the mutagenic activity of isovelleral (**1**).

Table 1. The IC_{50} values of (+)-**1**, (−)-**1**, (−)-**2**, and (+)-**2** for the inhibition of the specific binding of ^3H-SCH 23390 to the dopamine D1 receptor, and that of ^3H-resiniferatoxin to vanilloid receptors, respectively

Compd	IC_{50} (μM) ± SEM (n = 3) Dopamine D1	Vanilloid
(+)-**1**	0.29±0.01	2.7±0.3
(−)-**1**	0.48±0.01	2.4±0.6
(−)-**2**	0.26±0.02	1.3±0.4
(+)-**2**	2.26±0.5	0.9±0.2

The affinities of (+)-**1**, (−)-**1**, (+)-**2**, and (−)-**2** for the dopamine D1 receptor and the vanilloid receptor, tested as their ability to inhibit the binding of the ligand ^3H-SCH 23390 to the dopamine D1 receptor and of ^3H-resiniferatoxin to the vanilloid receptor, are shown in Table 1. The difference between (+)-**1** and (−)-**1** with respect to affinity for the dopamine D1 receptor is only small, although clearly significant. (−)-**2** is as potent as (+)-**1**, which is in agreement with the results obtained in a previous investigation,[7] but almost 10 times more potent than (+)-**2**. No significant difference in the affinity for the vanilloid receptor between the couples (+)-**1**/(−)-**1** and (+)-**2**/(−)-**2** could be observed, although there is a difference between **1** and **2**.

Discussion

It has been suggested that the natural unsaturated dialdehydes have an ecological function as repellents or antifeedants.[3,4,8] Their pronounced pungency, which at least is responsible for the antifeedant activity towards mammals,[4] was recently suggested to be caused by the affinity of the unsaturated dialdehydes to the vanilloid receptor.[18] This receptor also represents the shared target for two other classes of naturally occurring pungent compounds, exemplified by capsaicin (the

irritant substance in *Capsicum*) and resiniferatoxin (the active constituent in *Euphorbium* species). It is also believed that acid is painful due to the activation of vanilloid receptors. When comparing the pungency to the human tongue of (+)-**1** and (−)-**2** with their affinity to the vanilloid receptor, the latter is approximately five times more potent in both assays.[18] Interestingly, the pungency of (−)-**1** is comparable to that of (−)-**2** (lowest amounts on a filter disk that provoke a pungent sensation to human tongues are 100 ng) while it takes approximately 500 ng of (+)-**1** or (+)-**2** to give the same effect. However, the affinity within the couples (+)-**1**/(−)-**1** and (+)-**2**/(−)-**2** for the vanilloid receptor does not differ significantly, and it should be noted that the binding to the vanilloid receptor reflects rat spinal cord preparations. The discrepancy may reflect species-related differences in the pharmacology of vanilloid receptors. The unsaturated dialdehydes have been shown to have a selective affinity for the dopamine D1 receptor in the CNS, but not to other CNS receptors.[7] As can be seen in Table 1, there is a small but significant difference between (+)-**1** and (−)-**1** with respect to affinity for the dopamine D1 receptor, while (−)-**2** is almost 10 times more potent than (+)-**2**. Besides being less potent, (+)-**2** also differs from the other isovelleraloids by not fully inhibiting the binding of ^{3}H-SCH 23390, suggesting that (+)-**2** has different affinities for the various dopamine D1 receptor subtypes, a finding that warrants further investigations.

Conclusion

In conclusion, it appears as if the unsaturated dialdehydes may give rise to bioactivity both by a general mechanism depending on the reactivity of the dialdehyde functionality towards bionucleophiles, resulting in cell toxicity, and by binding more selectively to certain cellular structures (e.g., DNA and receptors). In the latter case the absolute as well as the relative stereochemistry of the isovelleraloids has an impact on their ability to interact with biomolecules, and identification of the factors determining such binding will be subject to further studies.

Experimental

General procedures

TLC analyses were made on Merck DC-Alufolien Kieselgel 60 F_{254} SiO_2 plates, visualized by spraying with anisaldehyde/sulphuric acid and warming to 120 °C. EIMS spectra (direct inlet, 70 eV) were recorded with a JEOL SX102 spectrometer, and the NMR spectra (in $CDCl_3$) with a Bruker ARX 500 spectrometer at 500 MHz (^{1}H) and 125 MHz (^{13}C), and with a Bruker DRX 400 spectrometer at 400 MHz (^{1}H) and 100 MHz (^{13}C). The chemical shifts are reported in ppm with the solvent signals (δ_H = 7.26 and δ_C = 77.0) as reference. The melting points (uncorrected) were determined with a Reichert microscope, and the optical rotations were measured with a Perkin–Elmer 141 polarimeter at 22 °C. All preparative, centrifugally accelerated, radial TLC separations were carried out on a Chromatotron,[19,20] model 7924T (TC Research, Huntington, York, UK), with rotors coated with a 2 mm layer of Silica gel 60 PF-254 (with calcium sulfate), E. Merck, item no. 7749. MPLC separations were performed on a Merck Lobar® Größe A, LiChroprep® Si 60 pre-packed silica (particle size, 40–63 mm) column. (+)-Isovelleral (**1**) was isolated from fruit bodies of *L. vellereus*.[3] (−)-Menthoxyacetyl chloride was prepared by treating (−)-menthoxyacetic acid with thionyl chloride.[21] Compound (−)-**2** was prepared from (+)-isovelleral (**1**) by thermal rearrangement.[15]

Biological assays

The assays for antimicrobial,[22] cytotoxic,[23] phytotoxic,[22] and mutagenic activity,[24] pungency,[18] as well as affinity for the dopamine D1[25] and vanilloid[18] receptors, were essentially carried out as described previously.

Antimicrobial activity

The antimicrobial activity was determined in the serial dilution assay (concentration range 0.1–50 μg/mL, in steps of two), and repeated at least twice. The test organisms used were *Paecilomyces variotii*, *Penicillium notatum*, *Mucor miehei*, *N. coryli*, *B. brevis*, *B. subtilis*, *Sarcina lutea*, and *Enterobacter dissolvens*.

Cytotoxic activity

Cytotoxicity was assayed with B16-F1 (ATCC CRL 6323), BHK21 (ATCC CCL 10), and L1210 (ATCC CCL 219) cells, and measured in microtiter plates with 3×10^4 to 1×10^5 cells/mL. The concentrations of the tested compounds were 0.1–50 μg/mL, in steps of two.

Phytotoxic activity

Seeds of *Setaria italica* and *Lepidium sativum* were grown on paper disks in the presence of the assayed compounds (0.1–50 μg/disk, in steps of two), and the effect on the germination of the seeds was examined after 72 h.

Mutagenic activity

The Ames test was carried out as a pour-plate (plates containing 6 mL) assay without S9-mix with the strain TA98 and the tested compounds (0.1–50 μg/plate, in steps of two), and revertants were counted after 48 h. Daunomycin and ethyl methanesulfonate were used as control mutagens.

Pungency

The pungency of the compounds towards the human tongue was assayed by MJ and OS by keeping a paper disk impregnated with the tested compound (0.05, 0.10, 0.25, 0.50, and 1.0 μg) on the tip of the tongue for 1 min.

D1 receptor binding assays of the isovelleraloids

^{3}H-SCH 23390 [(R)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol, *N*-methyl-^{3}H, 2638 Gbq/mmol] was obtained from New England Nuclear, Du Pont, U.S.A. Male Wistar rats (weighing 200–230 g) were decapitated and the brain removed. Striata were dissected and rapidly homogenized (Ultra Turrax, 10 s) in buffer A (50 mM KH_2PO_4, pH 7.4) and centrifuged at 30,000 × *g* for 10 min. The pellet was resuspended in buffer A at a final concentration of 1 mg original tissue/mL. Twenty-five μL ^{3}H-SCH 23390 (final concentration 0.2 nM) were added to 1 mL aliquots of homogenate and incubated in the absence (control) or presence of isovelleral derivatives (concentration range 0.1–50 μM, in steps of two) for 60 min at 30 °C. Samples were rapidly filtered through Whatman GF/C filters and washed twice with 10 mL of buffer A. Non-specific binding was obtained in the presence of *cis*-(*Z*)-flupentixol (1 μM final concentration). All binding assays were done in triplicate.

Vanilloid receptor binding assays of the isovelleraloids

^{3}H-Resiniferatoxin (1369 Gbq/mmol) was obtained from NCI-FCRDC, Frederick, MD, U.S.A. Female Sprague–Dawley rats (weighing 200–250 g) were killed by cervical dislocation, and their trigeminal ganglia, along with the cervical section of the spinal cord, were collected in ice-cold buffer B (KCl 5, NaCl 5.8, $CaCl_2$ 0.75, $MgCl_2$ 2, sucrose 320, and HEPES 10 mM, pH 7.4). After disruption of the tissue, the supernatants obtained after centrifugation for 10 min at 1000 *g* (4 °C) were centrifuged for 30 min at 35,000 × *g* (4 °C), and the resulting high-speed pellets were resuspended in buffer B and stored at −70 °C until assayed. Binding assays were carried out in a final volume of 500 μL, containing buffer B, supplemented with 0.25 mg/mL bovine serum albumine, membranes (40–50 μg protein), ^{3}H-resiniferatoxin, and the isovelleral derivatives (concentration range 0.1–50 μM, in steps of 2). All binding assays were done in triplicate.

1a*S*-(1aα,3aβ,6aβ,6bα)3a,4,5,6,6a,6b-hexahydro-5,5,6b-trimethylcycloprop-[*e*]indene-1a,2(1*H*)-dimethyl-menthoxyacetate (4) and 1a*R*-(1aα,3aβ,6aβ,6bα)-3a,4,5,6,6a,6b-hexahydro-5,5,6b-trimethylcycloprop-[*e*]indene-1a,2(1*H*)-dimethyl-menthoxyacetate (5). Thompson and Heathcock's procedure[14] for the synthesis of racemic isovelleral was employed to obtain the racemic diol (±)-**3**. To a solution of (±)-**3** (307 mg) in pyridine (5 mL) an excess of (−)-menthoxyacetyl chloride (1 mL) was added. The solution was kept at room temperature for 2 h and then poured into ice H_2O and extracted with Et_2O. The organic phase was washed with H_2O and satd $NaHCO_3$, and was concentrated in vacuo. The crude diastereomeric mixture was subjected to centrifugal TLC fractionation on a chromatotron, followed by MPLC fractionation, using CH_2Cl_2:*n*-BuOAc 300:1 as the eluent in all separations. Using this procedure, 70 mg of **4** (lower R_f) and 224 mg of **5** (higher R_f) were obtained as colorless oils, along with 316 mg of mixed material. The recovered diastereomers **4** and **5** were >99% diastereomeric pure, as judged by ^{1}H NMR.

To a stirred solution of natural (+)-isovelleral (**1**) (13 mg, 0.057 mmol) in EtOH (1 mL) was added $NaBH_4$ (6 mg, 0.16 mmol) at room temperature. After 15 min all isovelleral was consumed. Acetone was added and the solution was stirred for an additional 5 min. The solvent was then evaporated. The residue was partitioned between Et_2O and water, the organic phase was washed with brine, dried, and concentrated to afford the crude diol (+)-**3**[26] (12 mg) as a white solid.

To a solution of crude diol (+)-**3** (12 mg) in pyridine (1 mL) an excess of (−)-menthoxyacetyl chloride (0.10 mL) was added. The solution was kept at room temperature for 2 h and then poured into ice H_2O and extracted with Et_2O. The organic phase was washed with H_2O and satd $NaHCO_3$, and was concentrated in vacuo. The crude product was purified by chromatography (SiO_2, heptane:EtOAc 19:1) to provide the diester (18 mg, 51%, two steps) as a colorless oil. The diester showed to be identical in all respects with compound **4**. No traces of the diastereomer **5** could be detected, according to ^{1}H NMR, establishing that natural (+)-isovelleral (**1**) is >99% enantiomeric pure. Furthermore, this established the absolute configurations of the diesters **4** and **5** to be 1a*R*-(1aα,3aβ,6aβ,6bα) for **5**, corresponding to (−)-isovelleral, and 1a*S*-(1aα,3aβ,6aβ,6bα) for **4**, corresponding to (+)-isovelleral (**1**). For **4**: $[\alpha]_D$ −48.3 (*c* 1.0, $CHCl_3$). MS [*m/z* (% rel. int.)]: 414 (M^+-$C_{12}H_{22}O_3$, 18), 218 (50), 202 (61), 201 (44), 200 (100), 185 (17), 139 (16), 122 (17), 83 (38), 69 (18). ^{1}H NMR: 5.28, bs, 1H; 4.73, d, *J* = 12.2, 1H; 4.70, d, *J* = 12.3, 1H; 4.57, d, *J* = 12.5, 1H; 4.14, dd, *J* = 16.1, *J* = 7.2, 2H; 4.05, d, *J* = 12.5, 1H; 4.04, dd, *J* = 16.1, *J* = 2.4, 2H; 3.14, dt, *J* = 10.6, *J* = 4.1, 2H; 2.43, m, 2H; 2.27, m, 2H; 2.06, dm, *J* = 12.3, 2H; 1.75, dd, *J* = 13.2, *J* = 7.8, 1H; 1.61, m, 6H; 1.37–1.22, m, 7H; 1.18, s, H_3; 1.00, s, H_3; 0.99, s, H_3; 0.94, d, *J* = 4.4, 1H; 0.92, d, *J* = 1.9, H_3; 0.90, d, *J* = 1.8, H_3; 0.89, s, H_3; 0.88, s, H_3; 0.78, d, *J* = 2.8, H_3; 0.77, d, *J* = 2.8, H_3; 1.00–0.78, m, 4H; 0.67, d, *J* = 4.4, 1H. ^{13}C NMR: 170.8; 170.5; 133.0; 131.2; 80.3; 80.3; 67.3; 66.2; 66.0; 65.9; 48.1; 48.0; 47.8; 44.8; 42.1; 39.9; 39.9; 38.3; 37.4; 34.4; 34.4; 31.8; 31.7; 31.5; 31.5; 27.4; 26.2; 25.4; 25.4; 23.9; 23.2; 23.2; 22.3; 22.3; 21.0; 21.0; 20.8; 16.2; 16.2. For **5**: $[\alpha]_D$ −73.3 (*c* 6.0, $CHCl_3$). MS [*m/z* (% rel. int.)]: 414 (M^+-$C_{12}H_{22}O_3$, 18), 218 (50), 202 (61), 201 (44), 200 (100), 185 (17), 139 (16), 122 (17), 83 (38), 69 (18). ^{1}H NMR: 5.28, bs, 1H; 4.74, d, *J* = 12.2, 1H; 4.68, d, *J* = 12.2, 1H; 4.55, d, *J* = 12.5, 1H; 4.13, dd, *J* = 16.2, *J* = 10.7, 2H; 4.06, d, *J* = 12.5, 1H; 4.06, dd, *J* = 16.1, *J* = 1.8, 2H; 3.14, dt, *J* = 10.6, *J* = 4.1, 2H; 2.43, m, 2H; 2.29, m, 2H; 2.05, m, 2H; 1.75, dd, *J* =

13.2, J = 7.9, 1H; 1.61, m, 6H; 1.38–1.22, m, 7H; 1.18, s, H_3; 1.00, s, H_3; 0.99, s, H_3; 0.94, d, J = 4.5, 1H; 0.91, s, H_3; 0.90, s, H_3; 0.89, d, J = 1.7, H_3; 0.88, d, J = 1.6, H_3; 0.79, d, J = 3.6, H_3; 0.77, d, J = 3.6, H_3; 1.00–0.79, m, 4H; 0.67, d, J = 4.5, 1H. ^{13}C NMR: 171.2; 170.8; 133.4; 131.7; 80.6; 80.6; 67.7; 66.5; 66.4; 66.4; 48.6; 48.5; 48.3; 45.2; 42.5; 40.4; 40.4; 38.7; 37.9; 34.8; 34.8; 32.3; 32.1; 31.9; 31.9; 27.9; 26.6; 25.8; 25.8; 24.3; 23.7; 23.7; 22.7; 22.7; 21.4; 21.4; 21.3; 16.7; 16.7.

(+)- and (−)-Isovelleral (1). Compound **5** (224 mg) was dissolved in a 5% KOH–MeOH solution (10 mL) and stirred for 1 h at room temperature. The solution was concentrated in vacuo, diluted with H_2O, and extracted with Et_2O. The organic phase was washed with hydrochloric acid (2 M) and brine, dried, and concentrated to afford 74 mg of crude (−)-**3**, $[\alpha]_D$ −1.1 (*c* 1.00, $CHCl_3$), having mass and ^{1}H NMR spectra identical to those reported[14] for the racemic diol (±)-**3**. The crude diol (−)-**3** was Swern oxidized according to Thompson and Heathcock's procedure[14] to give 70 mg (85%, two steps) of (−)-isovelleral (**1**) as a white solid. Recrystallisation in heptane gave white crystals with mp 102–104 °C; $[\alpha]_D$ −283 (*c* 1.6, $CHCl_3$), showing ^{1}H, ^{13}H NMR and mass spectra[15,26] identical in all respects to those of an authentic sample of (+)-isovelleral (**1**), its enantiomer. In comparison, natural (+)-isovelleral (**1**) showed $[\alpha]_D$ +276 (*c* 1.6, $CHCl_3$) in a subsequent measurement with the same cell and the same polarimeter.

Using the same synthetic procedure, hydrolysis of compound **4** yielded the diol (+)-**3**, which on subsequent Swern oxidation gave (+)-isovelleral (**1**). Its spectral data[15,26] were identical in all respects to those of an authentic sample of (+)-isovelleral (**1**).

[1a*S*-(1aα,3aα,6aα,6bα)]-3a,4,5,6,6a,6b-hexahydro-5,5,6b-trimethylcycloprop-[*e*]indene-1a,2(1*H*)-dicarboxaldehyde (+)-(2). A solution of (−)-isovelleral (**1**) (50 mg) in toluene (2 mL) was heated for 1 h at 180 °C under an N_2 atmosphere in a high-pressure glass tube. After cooling, the solution was concentrated, and the residue was purified by MPLC using toluene:MTBE (200:1) as the eluent. Besides the starting material (−)-**1** (18 mg, 36%, higher R_f), the isomer (+)-**2** (17 mg, 34%, lower R_f) was obtained. Recrystallisation in heptane gave white crystals with mp 73–75 °C; $[\alpha]_D$ +85.8 (*c* 0.6, $CHCl_3$), showing ^{1}H, ^{13}C NMR, and mass spectra[15] identical in all respects to those of an authentic sample of its enantiomer (−)-**2**.

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