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Synthesis of racemic and each enantiomer of 3-methylnonacosanol, a new plant growth regulator from *Lowsonia inermis*

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Abstract

An efficient synthesis of the title compound **I** in racemic and enantiomeric forms has been developed starting from 10-undecenoic acid. For the enantiomeric synthesis, a lipase catalyzed acylation strategy was employed to prepare the required methyl branched chiron which was subsequently derivatized to give the enantiomers of **I**. The plant growth regulatory (PGR) assay of **I**, carried out with hypocotyl cuttings of French beans (*Phaseolus vulgaris* L.) in Steinberg's nutrient medium revealed appreciable activity at a concentration of 2.5 ppm. The PGR activities of the compound both in racemic and enantiomeric forms were better than 1-triacontanol, the enantiomer, (*S*)-**I** being the best test candidate.

1. Introduction

The growing human demand for more food products caused by the population explosion and limited availability of cultivable land has provided an arduous task of increasing agricultural productivity. In this context, the importance of plant growth regulators (PGRs) has been well-recognized.[1] Consequently, a number of compounds such as 1-triacontanol[2] have acquired prominence owing to their growth regulating activities on different plants of agricultural and pharmacological interest. Earlier from our laboratory, some of the olefinic analogues of 1-triacontanol were found[3] to possess better plant growth regulating activity compared to 1-triacontanol. In this context, 3-methylnonacosanol **I**, a branched analogue of 1-triacontanol, seems an interesting candidate for PGR activity. Compound **I** has been isolated[4] from the Indian medicinal shrub *Lowsonia inermis* Linn., which is traditionally used for the treatment of jaundice, enlargement of the liver and spleen, leprosy and other skin diseases.[5] Thus, in addition to PGR assay,

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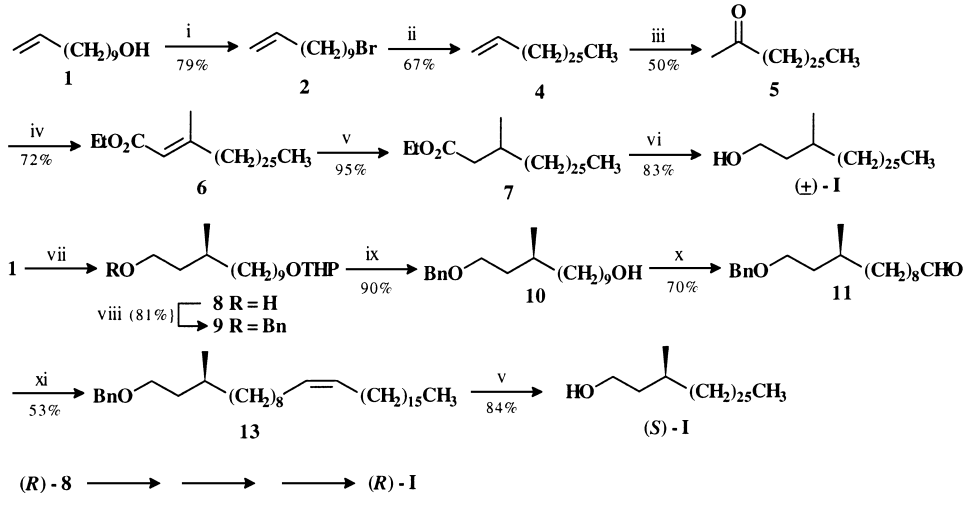
compound **I** warrants evaluation of other medicinal properties. For this, we have developed the first synthesis of compound **I** in enantiomeric and racemic forms and used them for their PGR activity vis-à-vis the stereochemistry. In this paper, the synthesis and the preliminary bioassay results are described. Details of the latter will be discussed elsewhere.

2. Results and discussion

2.1. Synthesis of (+)-*I*

The known alcohol **1**[6] was brominated with $Ph_3P/Br_2/Py$ to give compound **2**. Stearic acid was converted to the bromide **3**[7] via decarboxylative bromination by refluxing with HgO in the presence of Br_2. The Grignard reagent prepared from the bromide **2** was then coupled with **3** in the presence of Li_2CuCl_4 to afford the C_{28}-alkene **4**. For its conversion to the required ketone **5**, an oxymercuration strategy was envisaged. However, due to its poor solubility in the reaction medium, Hg^{2+}-catalyzed hydration[8] led to poor conversion. Hence, the alkene **4** was subjected to a Wacker oxidation[9] ($PdCl_2/CuCl_2$/DMF) to give the ketone **5**. Its Wittig–Horner reaction with triethyl phosphonoacetate led to the conjugated ester **6** which on catalytic hydrogenation over 10% Pd–C in ethanol furnished the ester **7**. Its LAH reduction provided the required alcohol (±)-**I** (Scheme 1) in good yield.



Scheme 1. (i) Ph_3P/Br_2/py/CH_2Cl_2; (ii) Mg/THF/Li_2CuCl_4/$CH_3(CH_2)_{16}Br$ (**3**); (iii) $O_2/PdCl_2/CuCl_2$/aqueous DMF/70°C; (iv) NaH/$(EtO)_2P(O)CH_2CO_2Et$; (v) H_2/10% Pd–C/EtOH; (vi) LAH/ether; (viii) lit.[10]; (viii) NaH/BnBr/Bu_4NI/Δ; (ix) MeOH/PTS; (x) PCC/CH_2Cl_2; (xi) Dimsyl ion/DMSO/$CH_3(CH_2)_{16}PPh_3Br$ (**12**)/THF

2.2. Enantiomeric synthesis of *I*

The target compound belong to the class of molecules which owe their chirality to the presence of methyl branching. Realizing the importance of this structural feature in several bioactive compounds, we have earlier designed[10] a new methyl branched chiron viz. 12-tetrahydropyranyloxy-3-methyldodecanol **8** and used it for the synthesis of several insect pheromones of agricultural importance. Both the enantiomers of compound **8** were synthesized in good enantiomeric purities via a chemoenzymatic strategy starting from the alcohol **1**. The chiron also seemed tailor-made for the present synthesis which

was executed as follows. For the synthesis, (*S*)-**8** was benzylated[11] with BnBr using NaH as the base in the presence of Bu_4NI to give (*S*)-**9**. Its depyranylation afforded the C_{13}-hydroxy-derivative (*S*)-**10** which was oxidized with pyridinium chlorochromate (PCC) to give **11**. Preparation of the target compound from the above chiron required addition of a suitable C_{17}-moiety to it. This was accomplished via a Wittig olefination. Thus, the required C_{17}-phosphonium salt **12** was prepared from the bromide **3**. Its reaction with (*S*)-**11** in the presence of dimsyl anion as the base gave the C_{30}-compound (*S*)-**13**. Its catalytic hydrogenation over 10% Pd–C in EtOH led to simultaneous debenzylation providing (*S*)-**I** in good yield.

The (*R*)-enantiomer of the target compound **I** was also synthesized using (*R*)-**8** as the starting chiron. The spectral data of all the synthetic samples were commensurate with those reported.[4] For assaying the enantiomeric purities of (*S*)-**8** and its (*R*)-antipode, the individual compounds were oxidized with pyridinium dichromate (PDC) in DMF and depyranylated to the corresponding acids. These were then converted to the diastereomeric amides with (*R*)-phenylethylamine analyzed by capillary GLC under the conditions specified in the experimental section. The ees of (*S*)- and (*R*)-**8** were found to be 96 and 97.7%, respectively. Based on these result, the ees of the synthetic (*S*)-**I** and its enantiomer should be at least 96%.

3. Experimental

All the boiling points are uncorrected. The IR spectra were scanned with a Perkin–Elmer spectrophotometer model 837. The ^1H NMR spectra were recorded in $CDCl_3$ with a Bruker AC-200 (200 MHz) instrument. The optical rotations were measured with a Jasco DIP 360 polarimeter. The GLC analyses were carried out with a Shimadzu GC-7A chromatograph fitted with a stainless steel column and flame ionization detector using 3% OV-17 (2 m×0.5 mm) column and a N_2 flow rate of 40 ml/min. Anhydrous reactions were carried out under Ar using freshly dried solvents. The organic extracts were dried over anhydrous Na_2SO_4.

*3.1. 1-Bromoundecene **2***

To a cooled (0°C) and stirred solution of PPh_3 (29.63 g, 11.29 mmol) in CH_2Cl_2 (50 ml) was added Br_2 (18.72 ml, 5.5 M in CCl_4, 10.35 mmol). After stirring for 0.5 h, the alcohol **1** (16.0 g, 9.41 mmol) and pyridine (8.4 ml, 10.35 mmol) in CH_2Cl_2 (20 ml) were added to the mixture which was gradually brought to room temperature and stirred for another 2 h. The mixture was concentrated in vacuo, the residue dissolved in hexane and passed through a pad (2 in.) of silica gel. The eluent was concentrated in vacuo to give the bromide **2**. Yield: 17.3 g (79%); IR: 3100, 1650, 1010, 910 cm^{-1}; ^1H NMR: δ 1.3 (br. s, 14H), 1.9–2.1 (m, 2H), 3.6 (t, *J*=6.5 Hz, 2H), 4.8–6.2 (m, 3H).

*3.2. 1-Bromoheptadecane **3***

To a refluxing mixture of stearic acid (36.0 g, 0.127 mol) and HgO (20.86 g, 0.096 mol) in CCl_4 (150 ml) was dropwise added Br_2 (20.27 g, 0.127 mol) so as to maintain a gentle reflux of the mixture. After the addition was over, the mixture was further refluxed for 1 h, brought to room temperature, filtered and the filtrate concentrated in vacuo. The residue was taken in hexane and washed with water and brine, dried and concentrated. The residue was purified by column chromatography (silica gel, hexane) to give the compound **3**. Yield: 32.0 g (79%), bp: 140–143°C/1 mm; IR: 2980, 2860 cm^{-1}; ^1H NMR: δ 0.9 (dist.

t, 3H), 1.25 (br. s, 30H), 3.4 (t, *J*=7.5 Hz, 2H). Anal. calcd for $C_{17}H_{35}Br$: C, 63.93; H, 11.05; Br, 25.02; found: C, 64.21; H, 10.88; Br, 25.27.

*3.3. 1-Octacosene **4***

A Grignard reagent was prepared from **2** (8.0 g, 0.034 mol) and Mg (0.91 g, 0.038 mol) in THF (30 ml). To a stirred and cooled (−5°C) solution of the above reagent was added Li_2CuCl_4 (2.0 ml, 0.1 M solution in THF) followed by **3** (9.57 g, 0.034 mol) in THF (40 ml). Stirring was continued for 3 h at the same temperature and at room temperature overnight. The mixture was poured into ice cooled water, the organic layer separated and the aqueous layer extracted with ether. The combined organic extract was washed with water, brine and dried. Removal of solvent followed by column chromatography (silica gel, hexane) of the residue yielded pure **4**. Yield: 7.87 g (67%); IR: 3090, 1660, 1020, 990, 910 cm^{-1}; ^1H NMR: δ 0.9 (dist. t, 3H), 1.25 (br. s, 48H), 1.9–2.2 (m, 2H), 4.8–6.2 (m, 3H). Anal. calcd for $C_{28}H_{56}$: C, 85.63; H, 14.37; found: C, 85.72; H, 14.49.

*3.4. 2-Oxooctacosane **5***

To a stirred mixture of $PdCl_2$ (0.289 g, 1.63 mmol) and $CuCl_2$ (0.28 g, 2.08 mmol) in aqueous DMF (1:10, 10 ml) was gradually added the alkenol **4** (4.0 g, 1.02 mmol), while bubbling oxygen continuously. After 1 h, the temperature was raised to 60–70°C and maintained for 1 h. It was poured into dilute HCl (2 N) and extracted with ether. The extract was washed with water, brine, dried and concentrated. The product was chromatographed (silica gel, 0–5% EtOAc–hexane) to furnish pure **5**. Yield: 2.1 g (50%); IR: 1720, 740 cm^{-1}; ^1H NMR: δ 0.9 (dist. t, 3H), 1.2–1.65 (m containing a br. s, 48H), 2.1 (s, 3H), 2.35 (t, *J*=6 Hz, 2H). Anal. calcd for $C_{28}H_{56}O$: C, 82.28; H, 13.81; found: C, 82.47; H, 14.01.

*3.5. Ethyl 3-methyl-2-nonacosenoate **6***

To a cooled (0°C) and stirred suspension of hexane-washed NaH (0.282 g, 50% dispersion in oil, 5.88 mmol) in THF (20 ml) was added triethyl phosphonoacetate (1.32 g, 5.89 mmol). After stirring for 1 h, compound **5** (2.0 g, 4.90 mmol) in THF (20 ml) was added to it at 0°C. Stirring was continued for 3 h at the same temperature and at room temperature overnight. The mixture was poured into ice cold water, the organic layer separated and the aqueous layer extracted with ether. The combined organic extract was washed with water, brine and dried. Removal of solvent followed by column chromatography (silica gel, 0–10% EtOAc–hexane) of the residue gave **6**. Yield: 1.69 g (72%); IR: 3005, 1720, 1650 cm^{-1}; ^1H NMR: δ 0.9 (dist. t, 3H), 1.18 (t, *J*=6 Hz, 3H), 1.3–1.6 (br. s, 48H), 2.1–2.3 (m, overlapped with a s at δ 2.12, 5H), 4.1 (q, *J*=5.5 Hz, 2H), 5.4 (s, 1H). Anal. calcd for $C_{32}H_{62}O_2$: C, 80.27; H, 13.05; Found: C, 80.12; H, 13.34.

*3.6. Ethyl 3-methylnonacosanoate **7***

A mixture of **6** (1.6 g, 3.35 mmol) and 10% Pd–C (0.05 g) in EtOH (20 ml) was shaken under a positive pressure of hydrogen till the required uptake of the gas. The mixture was passed through a pad (2″) of silica gel which was thoroughly eluted with ether. The organic extract was concentrated in vacuo to give the ester **7**. Yield: 1.5 g (95%); IR: 1740, 730 cm^{-1}; ^1H NMR: δ 0.88–0.95 (merged d and t, 6H), 1.16 (t, *J*=6 Hz, 3H), 1.3 (br. s, 50H), 1.5–1.64 (m, 1H), 2.21 (d, *J*=7 Hz, 2H), 4.15 (q, *J*=5.4 Hz, 2H). Anal. calcd for $C_{32}H_{64}O_2$: C, 79.93; H, 13.42; found: C, 80.08; H, 13.62.

3.7. (±)-3-Methylnonacosanol **I**

To a stirred solution of $LiAlH_4$ (0.12 g, 3.16 mmol) in ether (50 ml) was added the ester **7** (1.0 g, 2.08 mmol) in ether (50 ml). After stirring for 3 h, the excess hydride was decomposed with aqueous saturated Na_2SO_4, the supernatant organic layer decanted and the solid washed with ether. Concentration of the organic extract furnished pure (±)-**I**. Yield: 0.76 g (83%); IR: 3400, 730 cm^{-1}; ^1H NMR: δ 0.85–0.95 (m, 6H), 1.25 (br. s, 52H), 1.5–1.8 (m, 1H), 2.1 (s, D_2O exchangeable, 1H), 3.7 (t, *J*=6 Hz, 2H); MS: m/z 438 (M^+). Anal. calcd for $C_{30}H_{62}O$: C, 82.11; H, 14.24; found: C, 82.24; H, 14.11.

3.8. (3S)-12-Tetrahydropyranyloxy-1-benzyloxy-3-methyldodecane **9**

To a stirred suspension of NaH (0.25 g, 5.2 mmol, 50% suspension in oil) in THF (25 ml) was added the alcohol (*S*)-**8** (1.2 g, 4 mmol) and the mixture gently refluxed for 1 h. After the evolution of H_2 ceased, Bu_4NI (96 mg, 0.26 mmol) was added followed by benzyl bromide (0.89 g, 5.2 mmol) and the reaction mixture refluxed for 2 h. It was cooled to room temperature, poured into ice-water and extracted with diethyl ether. The ether layer was washed with water and brine and finally dried. Removal of solvent followed by column chromatography of the residue (neutral alumina, grade II, 0–10% EtOAc–hexane) afforded **9**. Yield: 1.26 g (81%); $[\alpha]_D^{25}$ +1.22 (*c* 1.18, $CHCl_3$); IR: 3060, 3030, 1620, 870, 820 cm^{-1}; ^1H NMR: δ 0.84 (d, *J*=6 Hz, 3H), 1.28 (br. s, 18H), 1.4–1.6 (m, 7H), 3.2–3.7 (m, 4H), 3.81 (t, *J*=6 Hz, 2H), 4.4 (s, 2H), 4.51 (br. s, 1H), 7.1–7.3 (m, 5H). Anal. calcd for $C_{25}H_{42}O_3$: C, 76.87; H, 10.84; found: C, 77.02; H, 10.72.

3.9. (3S)-1-Benzyloxy-3-methyldodecan-12-ol **10**

A solution of **9** (1.2 g, 3.1 mmol) in MeOH (30 ml) containing a catalytic amount of PTS was stirred for 6 h at room temperature. Most of the solvent was removed in vacuo, the residue taken in ether and the ether layer washed with aqueous 10% $NaHCO_3$, water and brine. After drying, the extract was concentrated in vacuo and the product obtained was chromatographed (silica gel, 0–15% ether–hexane) to give **10**. Yield: 0.85 g (90%); $[\alpha]_D^{25}$ +3.44 (*c* 0.8, $CHCl_3$); IR: 3400, 3060, 3030, 1620 cm^{-1}; ^1H NMR: δ 0.88 (d, *J*=6 Hz, 3H), 1.31 (br. s, partially D_2O exchangeable, 20H), 3.68 (t, *J*=6 Hz, 2H), 3.78 (t, *J*=6 Hz, 2H), 4.5 (s, 2H), 7.1–7.3 (m, 5H). Anal. calcd for $C_{20}H_{34}O_2$: C, 78.38; H, 11.18; found: C, 78.52; H, 11.02.

3.10. (10S)-12-Benzyloxy-10-methyldodecanal **11**

To a stirred suspension of PCC (0.84 g, 3.89 mmol) in CH_2Cl_2 (20 ml) was added **10** (0.8 g, 2.61 mmol) in one lot. After stirring for 3 h, when the reaction was complete (cf. TLC), the mixture was diluted with ether (30 ml) and the supernatant passed through a pad of silica gel. The eluent was concentrated to give **11** which was sufficiently pure (cf. TLC) and used as such for the next step. Yield: 0.56 g (70%); $[\alpha]_D^{25}$ −1.18 (*c* 0.78, $CHCl_3$); IR: 3030, 2725, 1730, 700 cm^{-1}; ^1H NMR: δ 0.90 (d, *J*=6 Hz, 3H), 1.2–1.6 (m, 17H), 2.3–2.5 (m, 2H), 3.78 (t, *J*=6 Hz, 2H), 4.5 (s, 2H), 7.2–7.4 (m, 5H), 9.7 (t, *J*=1.5 Hz, 1H).

3.11. Heptadecyltriphenylphosphonium bromide **12**

A mixture of **3** (6.38 g, 0.02 mol) and triphenyl phosphine (5.25 g, 0.02 mol) in CH_3CN (50 ml) was refluxed for 48 h. Most of the solvent was removed in vacuo, the residue thoroughly washed with

anhydrous ether and dried in vacuum over P_2O_5 to get **12** as a pale brown gummy mass which was used in the next step by making a 0.1 M solution in THF.

3.12. *(12Z,3S)-1-Benzyloxy-3-methylnonacos-12-ene* ***13***

To a stirred solution of dimsyl anion (2.13 mmol) in DMSO [prepared from NaH (0.102 g, 2.13 mmol, 50% suspension in oil) and DMSO (20 ml)] was added **12** (21.3 ml, 0.1 M in THF, 2.13 mmol) in THF. After stirring for 1 h, compound **11** (0.5 g, 1.64 mmol) in THF (30 ml) was added to it and the mixture stirred at room temperature for 48 h. It was poured into ice-water, the organic layer separated, the aqueous portion extracted with ether, the combined organic extract washed with water and concentrated after drying. The residue obtained was dissolved in a small amount of hexane, cooled to 0°C and filtered. The solid residue was washed with cold hexane and the combined filtrate concentrated in vacuo. After repeating the hexane dissolution process three times, the residue was chromatographed (silica gel, 0–5% EtOAc–hexane) to furnish pure **13**. Yield: 0.46 g (53%); $[\alpha]_D^{25}$ +2.48 (*c* 1.2, $CHCl_3$); IR: 3030, 1660, 720 cm^{-1}; ^1H NMR: δ 0.88–0.92 (m, 6H), 1.36 (br. s, 44H), 1.5–1.7 (m, 1H), 1.9–2.1 (m, 4H), 3.81 (t, *J*=6 Hz, 2H), 4.5 (s, 2H), 5.3–5.4 (m, 2H), 7.2–7.4 (m, 5H).

3.13. *(3S)-3-Methylnonacosanol* ***I***

Compound **13** (0.4 g, 0.8 mmol) was hydrogenated over 10% Pd–C (0.02 g) in EtOH (10 ml) and the product chromatographed (silica gel, 0–20% EtOAc–hexane) to give (*S*)-**I**. Yield: 0.28 g (84%); $[\alpha]_D^{25}$ +1.61 (*c* 0.95, $CHCl_3$).

3.14. *(3R)-12-Tetrahydropyranyloxy-1-benzyloxy-3-methyldodecanol* ***9***

$[\alpha]_D^{25}$ −1.07 (*c* 2.35, $CHCl_3$).

3.15. *(3R)-1-Benzyloxy-3-methyldodecan-12-ol* ***10***

$[\alpha]_D^{25}$ −3.59 (*c* 1.12, $CHCl_3$).

3.16. *(10R)-12-Benzyloxy-10-methyldodecanal* ***11***

$[\alpha]_D^{25}$ +1.25 (*c* 1.6, $CHCl_3$).

3.17. *(12Z,3R)-1-Benzyloxy-3-methylnonacos-12-ene* ***13***

$[\alpha]_D^{25}$ −2.15 (*c* 2.6, $CHCl_3$).

3.18. *(3R)-3-Methylnonacosanol* ***I***

$[\alpha]_D^{25}$ −1.43 (*c* 1.15, $CHCl_3$).

4. PGR bioassay

Following the known procedure,[12] the PGR assay of (±)-, (*S*)- and (*R*)-**I** were carried out with hypocotyl cuttings of French beans, *Phaseolus vulgaris* L. in Steinberg's nutrient medium.[13] The experiments were carried out with 8-day-old young, healthy and similar plants using nutrient medium of 1/4th strength. The full strength medium was not suitable to realize any PGR effect of the samples and standard. The assays were carried out using the medium as the control and 1-triacontanol as the standard. The samples were applied in acetone solution and tested at four different concentrations viz. 1, 2.5, 5 and 7.5 ppm. Required amount of acetone was also added to the control. The results were compared with those obtained from 1-triacontanol at the same concentration levels. The experiments were carried out with three replicates and the mean differences in shoot and root weights were used for estimating the activity. At a minimum concentration of 2.5 ppm, the samples and standard gave appreciable PGR activity. The (*S*)-isomer of **I** showed better activity compared to that of its enantiomer which was slightly more active than (±)-**I**. Most interestingly, all the test samples of **I** were more active than 1-triacontanol. Under the optimized conditions, application of (*S*)-**I** led to increase in yield of both the shoots (24.1±2.3%) and the roots (33.7±1.6%) as compared to those with the standard.

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