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THE ABSOLUTE CONFIGURATION EFFECT ON THE ACTIVITY OF THE AVOCADO ROOTING PROMOTER

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Key Word Index—*Persea americana*; Lauraceae; avocado; root promoter; activity; chiral synthesis; 16-heptadecyn-1,2,4-triol.

Abstract—16-Heptadecyn-1,2,4-triol is the most active component of the avocado rooting promoter (ARP). All four diastereoisomers of this compound have been synthesized. Their root promoting activity was determined over the physiologically active concentration range. It was found that the (2*R*,4*R*)-stereoisomer exerts a rooting activity similar to that of the extracted and purified compound from avocado tissues. The (2*R*,4*S*), and (2*S*,4*R*)-stereoisomers had lower activity and the (2*S*,4*S*)-stereoisomer had the lowest activity. It is concluded that the natural form, (2*R*,4*R*), acts in the rooting process either in its original structure or after reaction which does not alter its chiral centres.

INTRODUCTION

In a previous paper we described a native, non-auxinic rooting promoter extracted from various avocado tissues [1]. It was later found that the partly purified fraction contains four closely related compounds which were identified and their root promoting activity was determined [2]. It was found that 16-heptadecyn-1,2,4-triol (**I**) is the most active one. Ketones and substituted furanes which may be formed by oxidation of **I** were also isolated from the avocado tissues [3]. It was found that they have no rooting activity (unpublished data).

The purpose of the present study is to clarify the relation between the absolute configuration and the biological activity. All four possible stereoisomers of compound **I** were synthesized stereoselectively and their biological activity tested. The results will have theoretical and practical consequences.

RESULTS AND DISCUSSION

The structure of compound **II** has been determined by Kashman [3]. The chiral centres were determined by Sugiyama [4] to be (2*R*,4*R*)-16-heptadecen-1,2,4-triol (**5**). We have modified the synthesis of **II** to the synthesis of **I** in the following way. A Grignard condensation between a four carbon chain carrying two protected alcohols and a 13 carbon unit having a protected terminal acetylenic group, led in one step to the skeleton of the target in the proper oxidation state. The sequence of reactions employed for the preparation of the two units is described in Scheme 1. Having an (*R*)-centre in **11** enabled us to prepare by this route the two diastereomers **1** (2*R*,4*R*) and **2** (2*R*,4*S*) in a 43:57 ratio. The mixture was separated by chromatography to give pure **1** and **2**. The second pair of stereoisomers **3** (2*S*,4*R*) and **4** (2*S*,4*S*) were prepared by using **11** (*S*) as a starting material. The four new compounds were identified by spectroscopic methods and their purity was established by GC to be over 98%. The

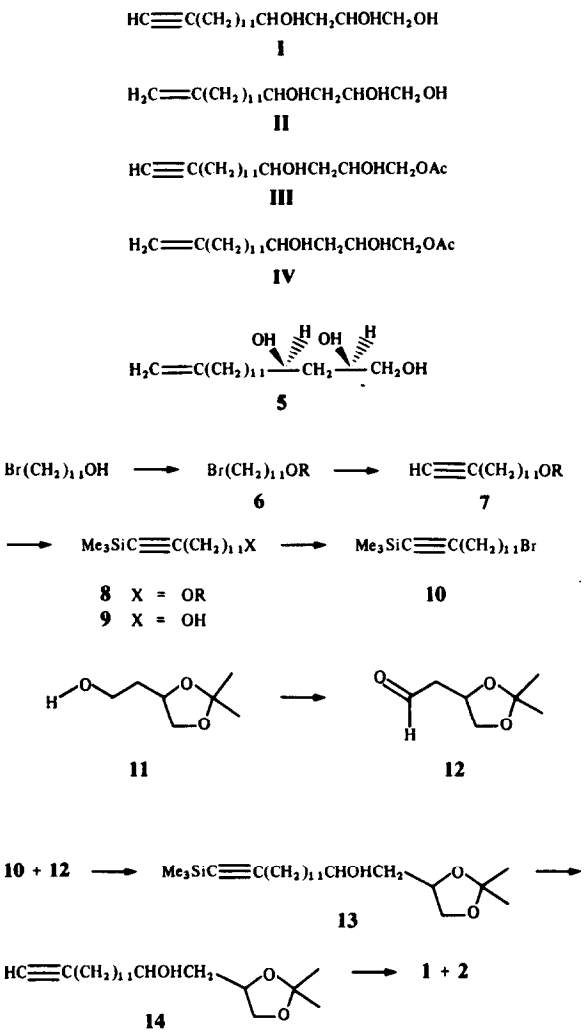

Table 1. Root promoting activity of the synthetic stereoisomers of compound I and the native compound (average no. of root per cutting of 3 replicates, per cent of control = 100%)

	Synthetic				Native
Conc.[M]	1 (2R,4R)	2 (2R,4S)	3 (2S,4R)	4 (2S,4S)	I (2R,4R)
0	100	100	100	100	100
5.10^{-6}	140	119	135	124	149
1.10^{-5}	212	153	169	113	240
2.10^{-5}	379	218	231	194	347

sequence of reactions carried out on **11** (*R*) are described in the Experimental, the same results were obtained for **11** (*S*). The stereoselective synthesis of all four stereoisomers of **I** has been carried out successfully. The new compounds were purified and tested by known bioassay [5, 6].

It was found that the absolute configuration of the chiral centres at C-2 and C-4 influence the activity of **I** as a rooting promoter. It can be concluded that the active compound that penetrates the plant is chiral. The active site in the plant can tolerate better variation in **I** at C-2 than at C-4. The possibility that **I** is oxidized to a ketone or furan derivative in the plant before it acts can be excluded. From a practical point of view it seems that maximum efficiency will be obtained by using **I** either from natural sources or a new inexpensive chiral synthesis.

EXPERIMENTAL

Silica gel Merck (250–400 mesh) kieselgel 60.

1-Tetrahydropyrane-11-bromo-undecane ether (**6**). *p*-Toluenesulphonic acid (0.2 g) and 10.1 g of 3,4-dihydropyrane (120 mmol) were added to soln of 25.1 g (100 mmol) 11-Bromo-1-undecanol (commercially available) in 100 ml dry Et_2O. The reaction was kept overnight at room temp. washed with 10% $NaHCO_3$, H_2O, and dried (Na_2SO_4). The solvent was removed by red. pres. to give, after chromatography over silica gel eluting with hexane, 30.8 g **6** in 90% yield.

1-Tetrahydropyrane-12-tridecyne ether (**7**). 1.5 equivalent of lithium acetylide was prepared according to ref. [7] in liq. NH_3. A soln of 10 g (30 mmol) of **6** dissolved in 10.8 g of HMPA (2 equiv.) were added to the acetylide soln at $-70°$ and stirred for 3 hr, the cooling bath was removed and the reaction was stirred overnight at room temp. A satd soln of NH_4Cl (50 ml) was added and the organic material extracted with hexane (4 × 30 ml), washed with H_2O and dried (Na_2SO_4). The solvents were removed by red. pres. to give 6.6 g **7** in 80% yield. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3320. ^1H NMR (60 MHz, CCl_4): δ4.4 (*br s*, 1H), 3.68–3.03 (*m*, 4H), 2.2 (*m*, 2H), 1.86 (t, 1H).

13-Trimethylsilyl-1-tetrahydropyrane-12-tridecyne ether (**8**). A soln of *n*-BuLi 10.8 ml (2.3 mol in cyclohexane) was added to a soln of 6.5 g (23.6 mmol) **7** in 30 ml dry Et_2O at 0° and stirred at that temp. for 1 hr and 30 min at room temp. The reaction was cooled to 0° and 2.8 g (26 mmol) trimethylchlorosilane were added and stirred for 1 hr at 0° and then for 1 hr at room temp. H_2O (50 ml) was added and the reaction was extracted with Et_2O (3 × 30 ml), washed with brine (30 ml) and dried (Na_2SO_4). The solvent was removed by red. pres. to give 7.6 g **8** in 92% yield. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2178. ^1H NMR (60 MHz, CCl_4): δ4.4 (*br s*, 1H), 3.85–3.05 (*m*, 4H), 2.1 (*m*, 2H), 0.15 (*s*, 9H).

13-Trimethylsilyl-12-tridecyn-1-ol (**9**). Ether **8** (7.5 g, 21.6 mmol) was dissolved in 40 ml MeOH, 0.1 g *p*-toluenesulphonic acid were added and the soln was stirred overnight at room temp. The acid was quenched by solid $NaHCO_3$, the solids were removed by filtration and the solvent removed by red. pres. to give 5.1 g **9** in 90% yield. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2189, 3640. ^1H NMR (60 MHz, CCl_4): δ3.4 (*t*, 2H), 2.1 (*m*, 2H), 0.15 (*s*, 9H).

13-Trimethylsilyl-1-bromotridecyne (**10**). Methansulphonyl chloride 5.6 ml (24.3 mmol) was added during 12 min to soln of 5 g (18.9 mmol) of alcohol **9** and 3.37 ml (24.26 mmol) of triethylamine in 25 ml dry CH_2Cl_2 at 0°. The reaction was stirred at 0° for 30 min and 2 hr at room temp., H_2O (25 ml) was added and the organic layer sepd. The organic phase was washed with 1 M HCl (20 ml), brine (20 ml), and dried (Na_2SO_4). The solvent was removed at red. pres. to give crude mesylate in 95% yield. The crude mesylate was dissolved in 30 ml dry THF and 2.9 g (33 mmol) of dry LiBr were added at 0°. The cooling bath was removed and the reaction refluxed for 2 hr. H_2O (30 ml) was added, the solvents removed by red. pres. The organic material was extracted by hexane (3 × 30 ml), washed with brine (20 ml) and dried (Na_2SO_4). The solvent was removed by red. pres. to give 4.5 g **10** in 73% yield. ^1H NMR (60 MHz, CCl_4): δ3.2 (*t*, 2H), 2.08 (*m*, 2H), 1.9 (*t*, 1H), 0.15 (*s*, 9H). HRMS $[M]^+$ (Calc. for $C_{16}H_{31}{}^{79}BrSi$: 330.1378, found: 330.1376).

(4R)-2,2-Dimethyl-1,3-dioxalane-4-ethanol (**11**). *p*-Toluenesulphonic acid (0.2 g were added to soln of 27 g (0.26 mmol) of (2*R*)-1,2,4-butanetriol (commercially available) in 350 ml Me_2CO. The soln was kept at room temp. over night, the acid was quenched by $NaHCO_3$ (s) and the solids removed by filtration. The solvent was removed by red. pres. and the crude distilled (bp 105–110° at 25 mmHg) to give 28 g **10** in 74% yield. ^1H NMR (60 MHz, CCl_4): δ4.3–3.23 (*m*, 5H), 1.73 (*q*, 2H), 1.34 (*s*, 3H), 1.3 (*s*, 3H).

(4R)-2,2-Dimethyl-1,3-dioxalane-4-ethanal (**12**). Oxally chloride 1.53 g (21 mmol) was dissolved in 25 ml dry CH_2Cl_2 and the soln was transferred to a three-necked flask equipped with thermometer and two dropping funnels. The soln in the three-necked flask was cooled to $-60°$, soln of 1.9 g DMSO (10.1 mmol) in 5 ml CH_2Cl_2 was added during 5 min and stirred for 10 min at $-60°$. The second soln, 1.5 g (10.1 mmol) of alcohol **11** in CH_2Cl_2, was added keeping the temp. below $-50°$, the mixture was stirred for 15 min and 1.1 g (55 mmol) ethyl diisopropyl amine was added and the cooling bath was removed. H_2O (30 ml) was added at room temp. and the organic material extracted by CH_2Cl_2 (3 × 10 ml). The organic phase was washed with cold 10% HCl, H_2O and dried over Na_2SO_4. The solvent was removed at red. pres. to give 1.3 g of crude **12** which was purified over silica gel column using hexane–CH_2Cl_2 (8:1) as eluant to give 1.2 g of **12** in 76% yield. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1735. ^1H NMR (60 MHz, CCl_4): δ9.28 (*t*, 1H), 4.43–3.86 (*m*, 2H), 3.4 (*dd*, 1H), 2.6 (*m*, 2H), 1.33 (*s*, 3H), 1.3 (*s*, 3H).

(2R,4RS) 1,2-Acetonide-17-trimethylsilyl-16-heptadecyn-1,2,4-triol (**13**). Grignard reagent was prepared as usual from 1 g bromide **10** (3.06 mmol) and 90 mg Mg in 5 ml dry Et_2O. The reagent was cold to 0° and soln of 445 mg of **12** (3.09 mmol) in 2 ml of Et_2O was added drop wise. The reaction was stirred over night at room temp., 5% NH_4Cl (20 ml) were added. The organic material was extracted by Et_2O (3 × 10 ml), washed with H_2O (15 ml) and dried over Na_2SO_4. The solvent was removed and the crude was purified over Florisil column using hexane–CH_2Cl_2 (2:1) as eluant to give 780 mg of **13** in 65% yield. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3520, 2180, 1380. ^1H NMR (400 MHz, $CDCl_3$): δ4.08 (*dd*, 1H), 3.81 (*m*, 1H), 3.55 (*dd*, 2H), 2.18 (*t*, 2H), 1.66 (*m*, 2H), 1.42 (*s*, 3H), 1.36 (*s*, 3H), 1.26 (*wm*, 18H), 0.15 (*s*, 9H). HRMS M_r $[M]^+$ Calc for $C_{23}H_{44}O_3Si$ 396.3123, found: 396.3120.

(2R,4RS) 1,2-Acetonide-16-heptadecyn-1,2,4-triol (**14**). To soln

of 700 mg of **13** (1.78 mmol) in 3 ml dry THF were added 2.2 ml tetrabutylammonium fluoride (1 mmol in THF) at room temp. and stirred for 10 min. H_2O (10 ml) was added and the product was extracted by Et_2O (4 × 10 ml), washed with brine and dried over Na_2SO_4. The solvent was removed by red. pres. to give 510 mg of oily compound **14** in 90% yield. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3520, 1320. ^1H NMR (400 MHz, $CDCl_3$): δ4.08 (*dd*, 1H), 3.81 (*m*, 1H), 3.55 (*dd*, 2H), 2.16 (*dt*, 2H), 1.93 (*t*, 1H), 1.66 (*m*, 2H), 1.42 (*s*, 3H), 1.36 (*s*, 3H), 1.26 (*wm*, 18H).

(2*R*,4*RS*)-16-*Heptadecyn*-1,2,4-*triol* (**1**+**2**). A soln of 500 mg **14** (1.56 mmol) and 50 mg *p*-toluenesulphonic acid in 5 ml of MeOH was kept for 10 hr at room temp. The acid was quenched with $NaHCO_3$ (s) and the solid were filtered, the solvent was removed by red. pres. to give 375 mg of solid mixture of **1**+**2** in 43:57 ratio. The isomers ratio was determined by GC, 25 m SE-54 capillary column 0.25 i.d., splitless injection, flame detector, injector temp. 190°, det. temp. 240°. Column flow 60 ml min^{-1}, head pres. 26 psi, air 400 ml min^{-1}, make-up gas 30 ml min^{-1}, Purge 3 ml min^{-1}, Purge time 3 min. Program: 3 min at 50°, heating 35 deg min^{-1} to 220°, hold 30 min. R_t for **1** 17.4 min, **2** 17.5 min. The alcohols were converted to the corresponding silyl derivatives (by *O*,*N*-bis (trimethylsilyl)-trifluoroacet amide) before the analysis. The synthetic **1** was found to be identical to **I** from natural sources by GC IR and NMR. Diastereomers **1** and **2** were separated over silica gel column, hexane–Me_2CO (2:1), **1** is less polar then **2**. The samples for activity test were over 98% pure according to GC analysis.

Biological test. Rooting of mung bean cuttings as described by Hess [5] and modified by Raviv *et al.* [6] was used in order to evaluate the rooting activity of the tested compounds. Concentrations were in the range of 1×10^{-6}–5×10^{-5} M. The lowest concentration is close to the actual concentration in the plant tissue (Raviv *et al.*, unpublished) and has no activity when applied exogenously. The highest concentration causes overdose symptoms. The presented concentration range is therefore 5 $\times 10^{-6}$ to 2×10^{-5} M. The bioassay was replicated × 3 with 20 cuttings per treatment in each replicate. The presented results are the average of the three experiments expressed as per cent of control = 100%. Actual number of roots in the water control were 5–6 roots/cutting with s.e. lower than 0.35 in all cases.

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