(R)-(-)-(E)-2,6-Dimethyl-3,7-octadiene-2,6-diol 6-O-β-D-Glucopyranoside: Natural Precursor of Hotrienol from Lulo Fruit (*Solanum vestissimum* D.) Peelings†

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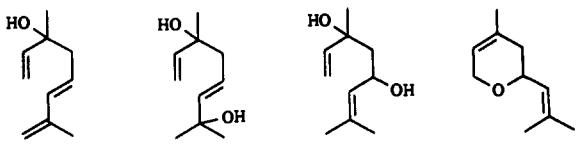
A glycosidic fraction was obtained from a methanolic extract of lulo fruit (*Solanum vestissimum* D.) peelings by Amberlite XAD-2 adsorption and methanol elution. Using preseparation by rotation locular countercurrent chromatography, subsequent acetylation, and liquid chromatographic purification, a glycoside was isolated in pure form whose structure was elucidated by DCI-MS and NMR analyses to be the 6-O-linked β-D-glucopyranoside of (R)-(-)-(E)-2,6-dimethyl-3,7-octadiene-2,6-diol.

INTRODUCTION

(E)-2,6-Dimethyl-1,3,7-octatrien-6-ol (hotrienol) (1), with a very sweet and flowery flavor (Nakatani et al., 1969), is a well-known constituent of the leaf oil of *Cinnamomum camphora* Sieb ("ho-oil") (Yoshida et al., 1969). It has also been found in a large number of other natural tissues, such as tea (Nakatani et al., 1969; Yamanishi et al., 1970), grapes and wines (Schreier et al., 1974; Bayonove et al., 1976; Rapp and Knipser, 1979), passion fruit (Engel and Tressl, 1983), elderberry flowers (Eberhardt and Pfannhauser, 1985), *Achillea ligustica* (Bruno and Herz, 1988), and papaya fruit (Winterhalter et al., 1986). The idea that 1 is not a natural product, but formed from the odorless (E)-2,6-dimethyl-3,7-octadiene-2,6-diol (2) under acidic conditions, has been advanced several times (Rapp and Knipser, 1979; Etoh et al., 1980; Williams et al., 1980).



1 2 3 4

As an additional precursor of 1 the allylic rearranged diol 3, a constituent of grapes (Strauss et al., 1988), has also been described. Thermal treatment of 3 at pH 3 revealed the formation of 1 together with neroloxide (4) via the diol 2 (Kitahara et al., 1980). Thus, whenever 4 occurs with hotrienol (1), a situation arises such as that found in tea flavor (Renold et al., 1974), grapes and wines (Rapp and Knipser, 1979; Williams et al., 1980), and pomaces (Etiévant and Bayonove, 1983), and one can assume the diols 2 and 3 are precursors.

Recently, the diol 2 has been detected as a major glycosidically bound constituent of lulo fruit (*Solanum vestissimum* D.) peelings (Suárez et al., 1991). In this paper, we describe for the first time the isolation and characterization of a glycoconjugate of 2 as a natural hotrienol (1) precursor from lulo fruit peelings.

† Dedicated to Prof. Dr. C. H. Brieskorn on the occasion of his 80th birthday.

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EXPERIMENTAL PROCEDURES

Chemicals. All commercial chemicals used were of analytical grade quality. Solvents were redistilled before use.

Fruits. Fresh lulo fruits (*S. vestissimum* D.) came by air freight from Virolin, Santander, Colombia. Pulp and peelings were separated.

Isolation of a Glycosidic Extract. After 10 kg of peelings was mixed with 10 L of methanol and macerated (adjusted to pH 7) at ambient temperature overnight, a clear extract was obtained by centrifugation (5000*g*, 30 min). Methanol was removed under reduced pressure (rotavapor). The aqueous residue was extracted three times with 100 mL of diethyl ether to separate free volatiles and then applied to an Amberlite XAD-2 column (25 × 900 mm, 10 mL/min) (Gunata et al., 1985). After the column was washed with 3000 mL of distilled water, a glycosidic extract was obtained by eluting with 1 L of methanol. The methanol eluate was concentrated under reduced pressure to dryness (rotavapor) and redissolved in 20 mL of citrate–phosphate buffer (pH 5) (yield, 9 g). Remaining volatiles were removed by diethyl ether extraction.

Rotation Locular Countercurrent Chromatography (RLCC). Four milliliters of the glycosidic extract was prefractionated using RLCC. The apparatus (Eyela RLCC, Tokyo Rikakikai Co.) was operated in the ascending mode employing a solvent system made from the two phases produced by mixing $CHCl_3$–MeOH–H_2O (7:13:8) with the more dense, less polar layer used as stationary phase. The flow rate was 1 mL/min, rotation speed 80 rpm, and slope 25°. Fifty-eight 10-mL fractions were separated and were analyzed by TLC using SiO_2GF_{254} (Merck, Darmstadt) as stationary phase and the less polar RLCC layer as mobile phase (detection, 254 nm and vanillin/H_2SO_4). RLCC fractions 1–24, 25–31, 32–37, 38–43, 44–52, and 53–58 were pooled, resulting in combined fractions I–VI, respectively. The presence of the aglycon moiety in these fractions was checked by (i) enzymatic and (ii) acid hydrolyses. The aglycon 2 was detected in fraction III.

Acetylation of RLCC Fraction III. The combined fractions III of five RLCC separations were concentrated under reduced pressure to dryness. The dry residue (150 mg) was acetylated by stirring with 5 mL of acetic anhydride and 300 mg of 4-(dimethylamino)pyridine in 5 mL of pyridine at ambient temperature overnight. After

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addition of 10 mL of ice water, extraction was performed three times with 100 mL of diethyl ether. The organic phase was extracted with 100 mL of 0.1 N HCl, neutralized with water, dried over anhydrous sodium sulfate, and concentrated in vacuo to 1 mL.

Flash Chromatograpy. The acetylated glycosidic fraction III was subjected to flash chromatography (Still et al., 1978) using a 10 × 400 mm glass column filled with silica gel (activity grade II, 0.032–0.063 mm; Merck). Elution with diethyl ether under N_2 pressure (20 mL/min) led to separation of 44 10-mL fractions. After check by TLC (cf. above), fractions 11–14 were combined and concentrated in vacuo to 1 mL for subsequent HPLC purification. Fractions 1–10 and 15–44 were discarded.

Preparative HPLC. The combined fractions 11–14 obtained by flash chromatography were finally purified by preparative HPLC using a 16 × 250 mm SiO_2 Lichrospher 60 column (5 μm; Knauer, Berlin). Elution was performed with diethyl ether (10 mL/min, detection 200 nm).

Deacetylation. After the addition of 1 mg of acetylated glycoside to a solution of 12 mg of sodium methylate in 5 mL of methanol and stirring overnight, 50 mg of Dowex 50-WX8 (20–50 mesh, H^+ form) was added. After 30 min, the exchanger was filtered off, the solvent removed under reduced pressure (rotavapor) to dryness, and the residue taken up in 5 mL of distilled water.

Enzymatic Hydrolysis. Five hundred micrograms of the deacetylated glycoside was dissolved in 50 mL of 0.2 M citrate–phosphate buffer (pH 5.0) and the solution incubated with 300 μL of Rohapect D5L (Röhm, Darmstadt) at 37 °C overnight. The liberated aglycon was extracted with diethyl ether; the organic phase was dried over anhydrous sodium sulfate and carefully concentrated to approximately 0.2 mL by a Vigreux column (45 °C) for subsequent HRGC and HRGC–MS analyses.

Acid Hydrolysis. A solution of 500 μg of deacetylated glycoside in 100 mL of distilled water (pH 3.0) was subjected to simultaneous distillation–extraction (SDE) (Schultz et al., 1977) over 2 h. The organic phase was dried over anhydrous sodium sulfate and carefully concentrated to approximately 0.2 mL by a Vigreux column (45 °C) for subsequent HRGC and HRGC–MS analyses.

Chiral Analysis of (*E*)-2,6-Dimethyl-3,7-octadiene-2,6-diol (2). After deacetylation of isolated glycoside **2b** and liberation of **2** by enzymatic hydrolysis (cf. above), chirality evaluation was carried out by analytical HPLC using a 4 × 125 mm SiO_2 Lichrospher 60 column (5 μm; Knauer) with an on-line coupled chirospecific detector (ACS ChiraMonitor, range 2, time constant 0.2) as well as UV detection at 200 nm. Elution was performed with diethyl ether (1 mL/min).

Chiral Analysis of (*E*)-2,6-Dimethyl-1,3,7-octatrien-6-ol (Hotrienol) (1) and Neroloxide (4). Chirality evaluation of 1 and 4 was carried out by on-line coupled multidimensional gas chromatography (DB5/Lipodex C)–mass spectrometry (MDGC–MS) as recently described (Bernreuther and Schreier, 1991).

Capillary Gas Chromatography (HRGC). A Carlo Erba Fractovap 4160 gas chromatograph with FID equipped with a J&W fused silica DB-Wax capillary column (30 m × 0.259 mm i.d., film thickness 0.25 μm) was used. Split injection (1:50) was employed. The temperature program was 3 min isothermal at 50 °C, raised to 220 °C at 4 °C/min. The flow rate for the carrier gas was 1.8 mL/min He; the flow rate for the makeup gas was 30 mL/min N_2, and those for the detector gases were 30 mL/min H_2 and 300 mL/min air, respectively. Injector and detector temperatures were kept at 220 °C.

Table I. ^1H NMR Spectral Data of Isolated Compound 2b ($CDCl_3$, 400 MHz, Coupling Constants in Hertz, δ Relative to TMS)

atom	δ	signal	J
aglycon			
$H_3$1	1.47[a]	3H, s	
H3	5.75	1H, d	15.8
H4	5.57	1H, dt	15.8/6.6
H5a	2.21	1H, dd	13.9/6.6
H5b	2.38	1H, dd	13.9/6.6
H7	5.93	1H, dd	17.5/11
H8a	5.05	1H, obscured	
H8b	5.16	1H, dd	17.5/1.0
$H_3$9	1.49[a]	3H, s	
$H_3$10	1.19	3H, s	
sugar moiety			
H1′	4.53	1H, d	8.1
H2′	4.98	1H, dd	9.6/8.1
H3′	5.19	1H, dd	9.6/9.6
H4′	5.02	1H, dd	9.9/9.6
H5′	3.60	1H, ddd	9.9/5.6/2.4
H6a′	4.08	1H, dd	12.1/2.4
H6b′	4.19	1H, dd	12.1/5.6
acetates	1.96–2.07	15H, 5 s	

[a] Interchangeable values.

Capillary Gas Chromatography–Mass Spectrometry (HRGC–MS). A Varian Aerograph 3300 gas chromatograph with split injector (1:20) was combined by direct coupling to a Finningan MAT 44 mass spectrometer with PCDS data system. The same type of column as mentioned above for HRGC was used. The conditions were as follows: temperature program, 3 min isothermal at 50 °C, raised to 220 °C at 4 °C/min and kept at 220 °C for 10 min; carrier gas flow, 1.5 mL/min He; temperature of ion source and all connection parts, 200 °C; electron energy, 70 eV; cathodic current, 0.8 mA; mass range, 41–250. Volumes of 1 μL were injected.

Results of qualitative analyses were verified by comparison of HRGC retention (*Ri*) and mass spectral data with those of authentic reference substances.

Desorption Chemical Ionization Mass Spectrometry (DCI-MS). DCI-MS was carried out with a Finnigan MAT 90 mass spectrometer at 70 eV using ammonia as reactant gas and ion source temperature and pressure of 150 °C and 1.5 × 10^{-4} mbar, respectively, as well as a temperature gradient of 400 °C/min. Mass range was 60–900.

Nuclear Magnetic Resonance (NMR). NMR spectra were recorded on a Bruker WM 400 (400 MHz) spectrometer with $CDCl_3$ as solvent and Me_4Si as internal standard.

RESULTS AND DISCUSSION

In the aglycon fraction of lulo fruit (*S. vestissimum* D.) peelings obtained by enzymatic hydrolysis (Rohapect D5L) of a methanolic eluate from XAD-separated glycosidic fraction (Gunata et al., 1985), (*E*)-2,6-dimethyl-3,7-octadiene-2,6-diol (**2**) has been identified as a major constituent of several glycosidically bound terpenoids by HRGC and HRGC–MS (Suárez et al., 1991). Due to former investigations on acid-catalyzed degradation of **2** (Williams et al., 1980), lulo fruit peelings are a convenient source for the isolation and characterization of a glycosidically bound precursor of (*E*)-2,6-dimethyl-1,3,7-octatrien-6-ol (hotrienol) (**1**).

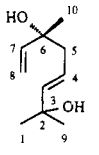
RLCC preseparation of a glycosidic extract provided fractions 32–37, in which **2** was detected as major aglycon after enzymatic treatment with Rohapect D5L. To prevent

Table II. ^{13}C NMR Spectral Data of Isolated Compound 2b and Reference Data of Aglycon 2 ($CDCl_3$, 100 MHz, δ · Relative to TMS)

atom	δ 2b	δ 2[a]
aglycon		
C1	26.78[b]	29.6[b]
C2	80.60[c]	70.4[c]
C3	138.00	142.1
C4	123.81	121.5
C5	43.40	44.9
C6	80.40[c]	72.6[c]
C7	141.91	144.6
C8	115.40	111.7
C9	27.09[b]	29.7[b]
C10	22.16	27.3
sugar moiety		
C1′	95.80	
C2′	71.31	
C3′	73.09	
C4′	68.76	
C5′	71.54	
C6′	62.31	
acetates		
CH_3CO (5×)	20.62–20.71	
CH_3CO (5×)	169.1–170.6	

[a] Etoh et al. (1980). [b,c] Interchangeable values.

degradation of the glycoconjugate of **2** in the following purification steps via dehydration, acetylation of the RLCC fractions was carried out with 4-(dimethylamino)pyridine as catalyst (Höfle et al., 1978). Subsequent flash chromatography on silica gel allowed the separation of a major glycoside, which was finally obtained in pure form by preparative HPLC on silica gel. The ^1H and ^{13}C NMR data of the isolated glycoside **2b** are represented in Tables



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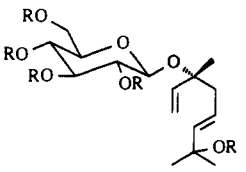

R = H 2a
R = Ac 2b

I and II. Due to the similar downfield shifts at carbon atoms 2 and 6, comparison of the ^{13}C NMR spectroscopic data of isolated glucoside **2b** with that of the corresponding aglycon **2** was not suitable to elucidate the position of glycosidic linkage (Table II). The position of glycosylation was established by means of a NOE experiment. Irradiation of protons of carbon 10 in **2b** at δ 1.19 ($CDCl_3$) produced a NOE on the anomeric proton of glucose, thus demonstrating that the glucose moiety was attached to carbon 6 of the aglycon. From these data the isolated compound was identified as a sugar conjugate from **2**, i.e., the pentaacetate of (*E*)-2,6-dimethyl-3,7-octadiene-2,6-diol 6-*O*-β-D-glucopyranoside (**2b**). The data recorded by DCI-MS were also in accordance with structure **2b** (Table III).

After deacetylation of **2b** and liberation of **2** by enzymatic hydrolysis with Rohapect D5L, the stereochemistry of the aglycon **2** was elucidated using HPLC on silica gel with

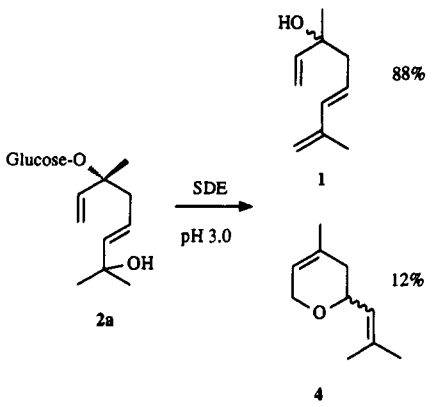

Figure 1. Acid-catalyzed degradation of glucoside **2a** (SDE, pH 3.0).

Table III. DCI Data of 2b (Reactant Gas Ammonia)

m/z	%	interpretation
560	100	$[M + NH_4]^+$
500	7	$[M - AcOH + NH_4]^+$
331	5	$[(hexose + 4\ acetyl - H_2O) + H]^+$
195	2	$[(aglycon + acetyl - H_2O) + H]^+$
135	4	$[(aglycon + acetyl - H_2O - AcOH) + H]^+$

chirospecific on-line detection (ChiraMonitor). According to the negative optical rotation of (*R*)-(*E*)-2,6-dimethyl-3,7-octadiene-2,6-diol (**2**) (Matsuura and Butsugan, 1968), the absolute configuration of the aglycon **2** was evaluated to be (*R*)-(−). The glucoside **2a** has not been described to date.

After deacetylation of the isolated glycoside **2b**, SDE treatment of **2a** at pH 3.0 yielded hotrienol (**1**) and neroloxide (**4**) as volatile degradation products in a 7:1 ratio (Figure 1). Previous degradation studies of **2** carried out by Williams et al. (1980) have revealed similar results. Chiral analysis of both hotrienol (**1**) and neroloxide (**4**) using MDGC–MS (DB5/Lipodex C) (Bernreuther and Schreier, 1991) showed in both cases the presence of racemic mixtures. The formation of racemic neroloxide (**4**) from enantiomerically pure **2** occurs during cyclization, while no conformation for attachment of the nucleophil is favored (Ohloff et al., 1964). Racemization of hotrienol (**1**) might be possible during the formation of a tertiary carbenium ion under SDE conditions (pH 3.0; 100 °C).

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