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The marine coelenterate *Echinopora lamellosa* (class Anthozoa, family Scleractinidae) was found to contain a number of unprecedented secondary metabolites which were isolated and identified as smilagenin (I), neodunol methyl ether (II), glycyrrhetic acid (III), 3β-acetoxyglycyrrhetic acid (IV), and 3β-acetoxy-11-deoxoglycyrrhetic acid (V). The structure of neodunol methyl ether was confirmed and its absolute configuration determined by the x-ray diffraction method.

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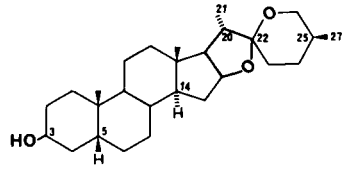
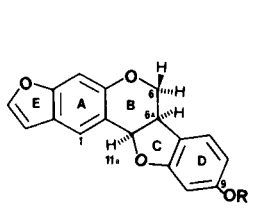
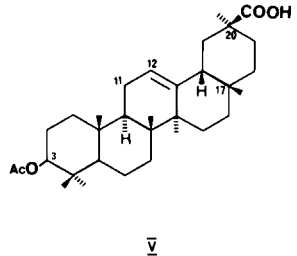
Marine invertebrates are known to produce a number of novel compounds with diversified carbon skeletons [1]. However, reports describing the occurrence of metabolites of terrestrial plants from marine sources are few [2,3]. In this communication we report the isolation of a number of secondary metabolites of the marine coral *Echinopora lamellosa* collected from the waters of Koralevu, Fiji [4]. Although these metabolites [smilagenin (I), neodunol methyl ether (II), glycyrrhetic acid (III), 3β-acetoxyglycyrrhetic acid (IV) and 3β-acetoxy-11-deoxoglycyrrhetic acid (V)] are common in plants and have been extensively investigated, their occurrence in a marine invertebrate is unprecedented. Smilagenin, along with glycyrrhetic acid and its 11-deoxo-derivative, constitute the major metabolites of *E. lamellosa* and were present in 0.98, 0.07 and 0.06% of the isopropanol extract of the coral. The occurrence of a pterocarpan (neodunol methyl ether, II) in *E. lamellosa* is also intriguing since pterocarpans are known to have antimicrobial activity and are produced by plants in response to bacterial and fungal infections [5]. Although neodunol (VI) has been isolated from *Neorautanenia edulis*, its methyl ether is known only synthetically [6,7]. This is the first report about the occurrence of neodunol methyl ether in nature. The significance of these metabolites in *E. lamellosa* is unclear. It remains to be seen whether these metabolites are produced by the coral or by some symbiont, although the possibility of their origin in the food web cannot be ruled out at this time.



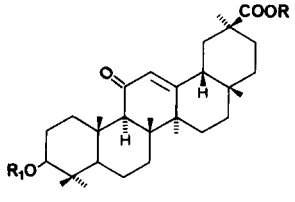
I



II R=CH_3
VI R=H



V



III = R=R_1=H
IV = H; R_1=$COCH_3$

The chloroform layer after concentration gave 4.76 g of a dark brown residue which upon chromatography followed by fraction pooling on the basis of tlc, gave five groups (A-E) of fractions. Combined fraction A consisted of a number of sterols, and was not investigated. Combined fraction B, after rechromatography, followed by crystallization, gave a colorless crystalline compound I (mp 185°), which was identified as (25*R*)-5β-spirostan-3β-ol (smilagenin) on the basis of its 400 MHz ^{1}H-nmr, ^{13}C-nmr and

Table 1

Comparison of the Selected ^{13}C-NMR Resonances of I, Smilagenin and Sarasapogenin

Carbon #	I	Smilagenin	Sarasapogenin
20	41.52	41.6	42.1
23	31.30	31.4	27.1
24	28.72	28.8	58.8
25	30.20	30.3	26.0
26	66.77	66.8	65.0
27	17.01	17.1	16.1

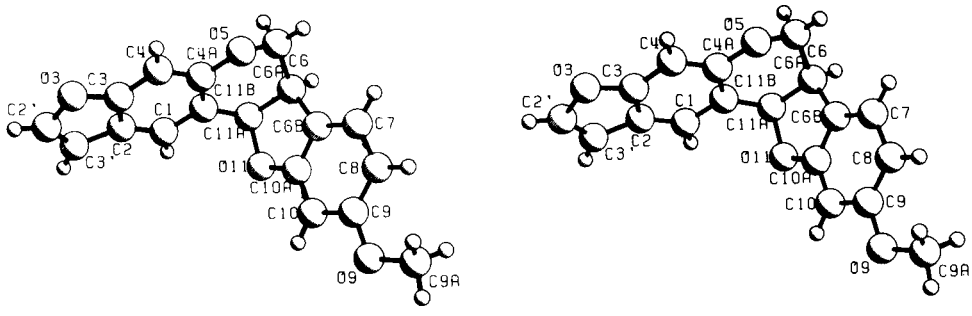


Figure 1. Stereoview of a single molecule of neodunol methyl ether (II).

mass spectral data. The possibility of this compound being sarasapogenin (25*S* isomer of smilagenin) was eliminated on the basis of the ^{13}C-nmr data (Table 1). The identity of I was confirmed by a direct comparison with an authentic sample of smilagenin [8].

Combined fraction C upon evaporation followed by hplc and crystallization gave light brown crystals II, mp 175°, $C_{18}H_{14}O_4$ (hrms). The 400 MHz ^{1}H-nmr spectrum (δ 6.17 and 6.64, 1H each C2′-H and C3′-H respectively) showed the presence of a furanopterocarpan (δ 2.69, 6a-H; 3.07, 6ax-H; 3.49, 6eq-H; and 4.94, 11a-H). The mass spectral (ms) fragments (m/z 158 and 148) also supported the presence of a furan moiety in II since 9-methoxypterocarpans are known to have base peak at m/z 148 [9,10]. On the basis of its ms and ^{1}H-nmr data, II was identified as 9-methoxyfuranopterocarpan (neodunol methyl ether). The structure of II was finally confirmed by a single crystal X-ray crystallography. A stereoview of the echinocarpin molecule is shown in Figure 1. The five ring system molecular backbone consists of a 6a-11a-dihydro-6*H*-benzofurobenzopyran nucleus II which assumes a folded conformation such that all the non-hydrogen atoms in the molecule lie approximately in two planes (one through the rings A, B and E and the other through the rings C and D) which are mutually inclined at about 80°. The two central heterocyclic rings (B and C) are *cis*-fused and the absolute configuration of the system is 6a,*R*:11a,*R*. The pyran ring is in a half-chair and the furan ring is in a slightly distorted envelope conformation.

The configuration 6a,*R*:11a,*R*, with *cis* B/C junction, is consistent with that assigned to a number of naturally occurring anti-fungal pterocarpans [11], but different from that given for phaseollin [12], a pterocarpan structure determined by X-ray diffraction (absolute configuration not determined).

In the *cis* configuration, the pyran ring of the benzofurobenzopyran system can be in two possible conformations [13]. In II the system assumes the one in which H(6a) is gauche to both the C(6) hydrogens [H(6a)-C(6a)-C(6)-H(61) = −70(2)°, H(6a)-C(6a)-C(6)-H(62) = 50(2)°]. The other possible conformation in which H(6a) is *trans* to one of the C(6) hydrogens has been observed in the case of phaseollin. Additionally, the conformation of the neodunol methyl ether molecule differs from that of phaseollin by a twist of about 47° about the bridge-head bond, C(6a)-C(11a). This is reflected in the overall molecular geometry of the two molecules. Phaseollin is relatively flat and shaped more like a propeller whereas II is V-shaped. The bond distances and bond angles in the present structure are normal. The molecular packing is compact, giving rise to a rather high density for the II crystals (1.477 gm cm^{-3}).

Table 2

Final Positional Parameters (×10^4) and Equivalent Isotropic Thermal Parameters (×10^3) for the Non-Hydrogen Atoms in Neodunol Methyl Ether (estimated standard deviations are in parentheses)

Atom	X	Y	Z	U
C1	7114(2)	2256(2)	5237(4)	17(1)
C2	7503(2)	2994(2)	7275(5)	18(1)
C2′	7872(2)	4281(2)	10324(5)	22(1)
C3	8641(2)	2851(2)	8899(4)	16(1)
C3′	7031(2)	3942(2)	8268(5)	21(1)
C4	9413(2)	2036(2)	8557(5)	16(1)
C4A	8991(2)	1307(2)	6564(4)	15(1)
C6	9591(2)	−63(2)	3673(4)	17(1)
C6A	8249(2)	−350(2)	2838(4)	16(1)
C6B	7677(2)	−1077(2)	4855(4)	16(1)
C7	8085(2)	−1985(2)	6054(5)	18(1)
C8	7329(2)	−2526(2)	7829(5)	18(1)
C9	6170(2)	−2133(2)	8378(4)	17(1)
C9A	5706(2)	−3561(2)	11257(5)	24(1)
C10	5742(2)	−1210(2)	7188(4)	17(1)
C10A	6508(2)	−715(2)	5407(4)	16(1)
C11A	7353(2)	570(2)	2896(4)	16(1)
C11B	7838(2)	1403(2)	4901(4)	16(1)
O3	8880(1)	3636(2)	10785(3)	19(1)
O5	9745(1)	478(2)	6370(3)	18(1)
O9	5366(1)	−2593(2)	10102(4)	22(1)
O11	6201(1)	174(0)	4001(3)	18(1)

Combined fraction D on rechromatography and crystallization gave colorless crystals of III [mp 280-285°, $C_{30}H_{46}O_4$ (hrms)]. The ^{1}H-nmr showed the presence of seven methyl singlets, an uncoupled vinylic proton (δ 5.68), a singlet at

Table 3

Hydrogen Parameters ($\times 10^3$) in Neodunol Methyl Ether

Atom	X	Y	Z	U
H1	633(2)	230(2)	417(5)	18(6)
H2′	793(2)	485(2)	1143(6)	30(7)
H3′	622(2)	428(2)	761(6)	27(7)
H4	1017(2)	195(2)	970(6)	19(6)
H61	1012(2)	−62(2)	399(5)	15(6)
H62	990(2)	37(2)	214(5)	19(6)
H6A	818(2)	−64(2)	81(6)	28(7)
H7	889(2)	−227(2)	568(5)	25(7)
H8	760(2)	−318(2)	863(5)	9(5)
H10	492(2)	−95(2)	749(5)	18(6)
H11A	711(2)	83(2)	95(5)	13(6)
H9A1	504(3)	−378(2)	1231(6)	29(7)
H9A2	650(2)	−353(2)	1249(6)	21(6)
H9A3	575(3)	−407(2)	964(6)	30(8)

Table 4

Bond Distances in Neodunol Methyl Ether
(estimated standard deviations are in parentheses)

C1 − C2	1.399(3)	C8 − C9	1.398(3)
C1-C11b	1.389(3)	C9 − C10	1.402(3)
C2 − C3	1.401(3)	C9-O9	1.372(3)
C2 − C3′	1.443(4)	C9a − O9	1.424(3)
C2′ − C3′	1.340(3)	C10 − C10a	1.383(3)
C2′ − O3	1.385(3)	C10a − O11	1.372(2)
C3 − C4	1.379(3)	C11a − C11b	1.508(3)
C3 − O3	1.369(3)	C11a − O11	1.480(2)
C4 − C4a	1.387(3)		
C4a − C11b	1.416(3)		
C4a − O5	1.373(3)		
C6 − C6a	1.518(3)		
C6 − O5	1.441(3)		
C6a − C6b	1.509(3)		
C6a − C11a	1.556(3)		
C6b − C7	1.380(4)		
C6b − C10a	1.393(3)		
C7 − C8	1.404(3)		

2.32 (1H) and a proton on an oxygen bearing carbon (δ 3.22). The presence of one αβ unsaturated carbon chromophore in III was evident from the uv (248 nm) and ir (1720 cm^{-1}) absorptions. On the basis of the multiplicities of resonances at δ 2.32 (1H, s, C9-H) and δ 5.68 (1H, s, C11-H) the carbonyl group (178.2 ppm) was fixed at C10. The presence of the 11-oxo moiety in III is also supported by the absence of significant m/z 246 and 207 fragments in the ms which constitute the major fragments in compounds of β-amyrin (olean-12-ene) class [14]. On the basis of spectral data III was identified as glycerrhetic acid. The geometry of C18-H was established to be β on the basis of the uv spectrum [15]. The structure of III was finally confirmed by a direct comparison with glycyrrhetic acid isolated from *Glycyrrhiza glabra* [16].

Fraction D also contained a minor compound IV which was isolated by repeated chromatography (mp 242°). The ^{1}H-nmr spectrum of IV was very similar to that of III, with the exception of a 3-proton singlet at δ 2.02 accompanied by a 1.27 ppm downfield shift in the C3-H resonance caused by the acetylation of the C3-OH group. The ms of IV ($C_{32}H_{48}O_5$, M^+) also showed the presence of an acetate in the molecule ($C_{30}H_{44}O_3$ = M^+-60), thus confirming the presence of an acetoxy group at C3. On the basis of the spectral data and correlation with III, compound IV was identified as 3β-acetoxyglycerrhetic acid, and was finally confirmed by the comparison with an authentic sample [17].

Rechromatography of combined fraction E gave colorless needles (V) [mp 245°, $C_{32}H_{50}O_4$]. The mass spectral fragmentation pattern [m/z 203 (100%) and 248 (38%)] indicated that V belongs to β-amyrins [18]. The ^{1}H-nmr spectrum of V showed the presence of seven methyl singlets along with resonances at δ 2.85 (1H, dd), δ 3.48 (3H, s), 4.46 (1H, dd) and 5.24 (1H, t). The positions and multiplicities of the downfield protons were very similar to what has been reported for abruslacton A and maytenfolic acid [18, 19]. On the basis of the spectral data, compound V was identified as 3β-acetyl-11-deoxoglycerrhetic acid.

EXPERIMENTAL

The coral *Echinopora lamellosa* (class Anthozoa, family Scleratinidae) was collected from the waters of Koralevu, Fiji (85 kg wet weight), and was immediately preserved in isopropanol. The filtered 2-propanol extract (35 ℓ) was concentrated under vacuum, followed by lyophilization to afford a residue (30 g). A portion of the residue (17 g) was triturated with hexane to remove lipoidal components. The hexane insoluble residue (12.9 g) was partitioned between carbon tetrachloride and 20% aqueous methanol. The polarity of the aqueous methanol layer was then increased to 35% followed by extraction with chloroform. The chloroform layer after evaporation gave 4.76 g of a residue which was chromatographed on a silica gel column (silica gel 60, E. Merck; 1.5 × 60 cm). The column was eluted with benzene:hexane (3:1 v/v) mixture (an FMI pump was used to deliver the solvent at ~40 psi). Fractions of 10 ml were collected and pooled on the basis of tlc [silica gel 60, E. Merck; solvent system: chloroform:methanol (95:5 v/v); fractions 3-5, 7-18, 21-29, 31-41, 42-49] to give combined fraction A-E. Melting points were recorded on a Fisher-Johns melting point apparatus and were uncorrected. Infrared spectra were recorded in carbon tetrachloride or in deuteriochloroform on a Perkin-Elmer Model 283 spectrophotometer. Low resolution EI mass spectra were recorded on a Finnigan 6000 equipped with a Model 6115 data system. High resolution mass spectra were recorded at the MIT Mass Spectrometry Facility. The 400 MHz ^{1}H-nmr spectra were recorded at the South Carolina Magnetic Resonance Laboratory, Columbia, South Carolina, and ^{13}C-nmr spectra were recorded on a Varian FT-80A spectrometer. Ultraviolet spectra were recorded on a Perkin-Elmer-Hitachi 200 spectrophotometer.

(25*R*)-Spirostan-3β-ol [Smilagenin (I)].

This compound was isolated from the rechromatography [silica gel 60 column (0.6 × 60 cm) solvent, benzene:hexane (3:1 v/v)] of the combined fraction B followed by crystallization from ether-methanol (168.2 mg), mp 185°; ir: (ν, cm^{-1}) 2910, 2830, 1450, 1240 and 1050; ms: m/z (% relative intensity) 416 (M^+, 10), 357 (5.3), 344 (12.6), 302 (24), 287 (21), 273 (37), 255 (12) and 139 (100); ^{1}H-nmr (400 MHz, deuteriochloroform): 0.73 (3H, s, C18-H), 0.76 (3H, d, J = 6.9 Hz, C21-H), 0.94 (3H, d, J = 6.9 Hz, C27-H), 0.95 (3H, s, C19-H), 3.35 (1H, t, J = 10.8 Hz), 3.44 (1H, m, $\omega^{1/2}$ = 7.2 Hz), 4.08 (broad s), and 4.37 (1H, m); ^{13}C-nmr (20 MHz, deuteriochloroform): 109.1, 80.8, 67.0, 66.7, 56.4, 41.5, 40.6, 40.2, 39.7, 36.4, 35.2

(2C), 33.4, 31.7, 31.3, 30.2, 29.8, 28.7, 27.7, 26.4 (2C), 23.8, 20.7, 17.0, 16.3, 14.3.

6a-11a-Dihydro-9-methoxy-6*H*-benzofuro[3,2-*c*]furo[3,2-*g*][1]benzopyran [Neodunol Methyl Ether (II)].

This compound was isolated from combined fraction C by hplc (Waters Model 201 equipped with a 250 nm uv detector, silica gel (5 μ) column (0.46 × 30 cm) solvent benzene:hexane (3:1 v/v) (9.74 mg), mp 175°; $C_{16}H_{14}O_4$ (hrms: found 294.0882; calcd. 294.0892); $[\alpha 0]_D^{25} = -217°$ (c = 0.145, chloroform); uv: (ν in methanol) 248 nm (log ϵ = 4.34); ir (carbon tetrachloride): 2850, 1620, 1500, 1280, 950 and 830 cm^{-1}; ^{1}H-nmr (400 MHz, benzene-d_6): δ 6.811 (1H, s), 6.74 (1H, s), 6.65 (1H, d, J = 2.1 Hz), 6.33 (1H, dd, J = 7.9, 0.8 Hz), 6.18 (1H, d, J = 2.1 Hz), 6.01 (1H, dd, J = 7.9, 2.2 Hz), 5.87 (1H, dd, J = 2.2, 0.8 Hz), 4.94 (1H, d, J = 7.1 Hz), 3.49 (1H, dd, J = 10.9, 5.2 Hz), 3.07 (1H, dd, J = 10.9, 10.7 Hz), 2.84 (3H, s), 2.69 (1H, ddd, J = 10.4, 7.1, 5.2 Hz). Irradiation of the δ 4.94 d (11a-H) collapsed the δ 2.69 ddd (6a-H) to a double doublet (J = 10.4 and 5.2 Hz), while irradiation of the δ 3.49 dd (6eq-H) collapsed the δ 3.07 dd (6ax-H) to a doublet (J = 10.7 Hz) and the 2.62 m (6a-H) to a double doublet (J = 10.4 Hz and an apparent 7.4 Hz). Conversely, irradiation at δ 3.07 changed the resonance at δ 3.49 to a doublet and irradiation at δ 2.69 collapsed the absorption at δ 4.94 to a singlet and also simplified the absorptions at δ 3.07 and 3.49 almost to doublets; ^{13}C-nmr (20 MHz, deuteriochloroform): 167.0, 161.2, 160.7, 155.6, 153.5, 145.0, 124.7, 122.9, 122.3, 119.1, 116.5, 106.4, 106.2, 99.8, 96.8, 79.3, 67.0, 39.9; high resolution ms: 294 (M^+, $C_{16}H_{14}O_4$), 279 ($C_{17}H_{11}O_4$), 261 ($C_{17}H_9O_3$), 171 ($C_{11}H_7O_2$), 158 ($C_{10}H_6O_2$), 148 ($C_9H_8O_2$, 100%) and 133 ($C_8H_5O_2$).

Crystals of II are monoclinic and belong to the space group $P2_1$ with a = 10.802(3), b = 13.218(4), c = 4.648(1) Å, β = 94.75(3) Å, V = 661.4 Å^3 and Z = 2 at 138K. The structure was solved by direct methods from 1425 reflections (2θ max = 53°) measured at 138°K on an Enraf-Nonius CAD-4 automatic diffractometer using graphite monochromatized MoKα radiation. All the hydrogen atoms were located from a difference Fourier map. The structure was refined by a full-matrix least-squares program with anisotropic thermal parameters for the non-hydrogen atoms and isotropic thermal parameters for the hydrogen atoms to a final R factor of 0.032 for all 1425 reflections. The absolute configuration of the molecule, as shown in Figure 1, was determined by the Bijvoet method using anomalous dispersion of CuKα radiation by the oxygen atoms.

3β-Hydroxy-11-oxo-olean-12-en-30-oic Acid [Glycyrrhetic Acid, (III)].

This compound was isolated from the combined fraction D by column chromatography [(silica gel 60 (230-400 mesh), column size 0.6 × 72 cm, eluted with a linear gradient of benzene in chloroform (0-40%)]. Fractions of 5 ml volume were collected and combined to give III (120 mg), mp 280-285°; ir (ν carbon tetrachloride): 3510, 1720, 1650, 1260 cm^{-1}; uv (λ, methanol): 248 nm; $C_{30}H_{46}O_4$: high resolution ms: found 470.34311, calcd. 470.33961; ^{1}H-nmr (400 MHz, deuteriochloroform): δ 5.68 (1H, s, C12-H), 3.21 (1H, dd, J = 10.7, 5.49 Hz, C3-H), 2.76 (1H, dd, J = 13.2 Hz, C18-H), 2.32 (1H, s, C9-H), 2.16 (2H, d, J = 19 Hz, C19-H), 1.34 (3H, s, C26-H), 1.18 (3H, s, C25-H), 1.12 (3H, s, C27-H), 1.10 (3H, s, C22-H), 0.97 (3H, s, C29-H), 0.804 (3H, s, C24-H), and 0.78 (3H, s, C23-H); high resolution ms: 470 (M^+, $C_{30}H_{46}O_4$), 455 ($C_{29}H_{43}O_4$), 437 ($C_{29}H_{41}O_3$), 424 ($C_{29}H_{44}O_2$), 409 ($C_{28}H_{41}O_2$), 391 ($C_{28}H_{39}O$), 303 ($C_{19}H_{27}O_3$), 302 ($C_{19}H_{27}O_3$), 262 ($C_{17}H_{26}O_2$), 165 ($C_{11}H_{17}O$), 150 ($C_{10}H_{14}O$), 135 ($C_9H_{11}O$).

3β-Acetoxy-11-oxo-olean-12-en-30-oic Acid [3β-Acetoxyglycyrrhetic Acid, (IV)].

This compound had mp 245°; ir (ν carbon tetrachloride): 1730, 1660, 1260 cm^{-1}; ms: m/z (% relative intensity) 512 (M^+, 9), 467 (15), 452 (11), 410 (22), 302 (33), 165 (38), 257 (9), 151 (33), 135 (100); ^{1}H-nmr (400 MHz, deuteriochloroform): δ 4.49 (1H, dd, J = 10.2, 4.6 Hz), 2.76 (1H, dd, J = 13.2 Hz), 2.32 (1H, s), 2.16 (2H, d, J = 19.0 Hz), 2.02 (3H, s), 1.34 (3H, s), 1.24 (3H, s), 1.12 (3H, s), 1.10 (3H, s), 0.85 (6H, s), 0.83 (3H, s).

3β-Acetoxyolean-12-en-30-oic Acid [3β-Acetoxy-11-dioxyglycyrrhetic Acid (V)].

This compound was isolated by column chromatography (0.5 × 6 cm packed with silica gel 60) of the combined fraction E. A linear gradient of benzene-chloroform (0-4% at 40 psi) was used as the eluting solvent. Crystallization from methanol gave colorless needles mp 242°; ir (ν) 1720, 1260 cm^{-1}; $C_{32}H_{50}O_4$ (hrms: found 498.3728, calcd. 498.3708); ms: 498 (M^+), 438 ($C_{30}H_{46}O_2$), 248 ($C_{16}H_{24}O_2$), 249 ($C_{16}H_{25}O_2$), 203 ($C_{15}H_{23}$; 100%), 189 ($C_{14}H_{21}$), 133 ($C_{10}H_{13}$); ^{13}C-nmr (80 MHz, deuteriochloroform): 143.5, 121.9, 78.4, 54.96, 49.13 (2 carbon), 48.78, 48.06, 47.28, 45.61, 41.33, 40.93, 38.88, 38.27, 38.18, 36.58, 33.45, 32.40, 32.17, 30.14, 27.48, 27.27, 26.32, 25.30, 22.94, 22.61, 17.91, 16.34, 15.01, and 14.70; ^{1}H-nmr (400 MHz, deuteriochloroform): 5.25 (1H, t, J = 3.3 Hz), 4.47 (1H, dd, J = 10.3, 4.9 Hz), 2.61 (1H, d, J = 13.5 Hz), 2.02 (3H, s), 1.95 (1H, d, J = 13.5 Hz), 1.102 (3H, s), 0.91 (3H, s), 0.90 (3H, s), 0.88 (3H, s), 0.84 (3H, s), 0.82 (3H, s), 0.71 (3H, s).

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