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Complete Assignment of the ^{1}H and ^{13}C NMR Spectra of a Sucrose Ester from *Euphorbia Lathyris L.*

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Abstract : *The detailed ^{1}H and ^{13}C NMR assignments of a novel sucrose isovaleryl ester isolated from the seed of Euphorbia Lathyris L., were achieved by one- and two-dimensional techniques. The new sucrose ester was characterized as an α-D-glucopyranoside, 3,4,6-tris-O-(3-methyl-1-oxobutyl)-β-D-fructofuranosyl, 2,6-bis(3-methylbutanoate); sucrose 4,7,8,11,12-pentaisovalerate by MS and NMR experiments.*

INTRODUCTION

Euphorbia Lathyris L. has been widely used to treat cancer, tumors and warts, and reports on its uses have appeared in many countries.[1-5] The methanolic extracts of this plant show a skin whitening activity. We have isolated a new sucrose isovaleryl ester and four diterpenes from the seeds of this plant.[1,4-8] In this paper, we report the complete ^{1}H and ^{13}C NMR chemical shift assignments using 2D-techniques for the new methanolic extract, sucrose 4,7,8,11,12-pentaisovalerate (**1**). NMR and mass spectra also determined the structures of the four diterpenes as 3-benzoate-5,10-diacetate-lathyrol (**2**), 3-benzoate-5,10-diacetate-7-hydroxy-lathyrol (**3**), 3-phenyl-acetate-5,10-diacetate-6,20-epoxy-lathyrol (**4**), and ingenol-20-palmitate (**5**).[4] Structures and numberings are shown in Fig. 1.

EXPERIMENTAL

Purification was performed on Merck (Darmstadt, Germany) Kiesegel 60 and 40 (70-230 and 35-70 mesh, respectively) silica gel chromatographic columns with filling amounts

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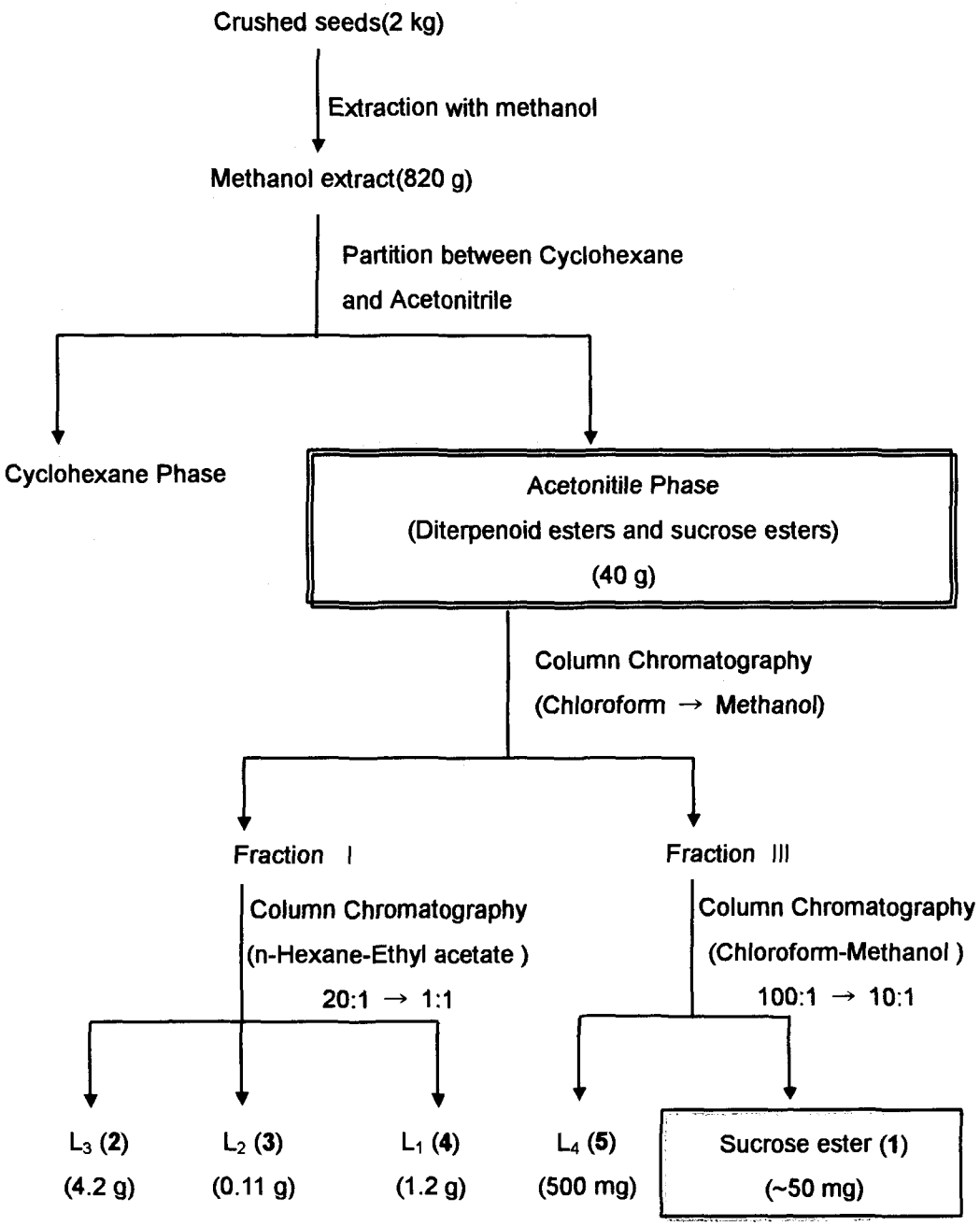
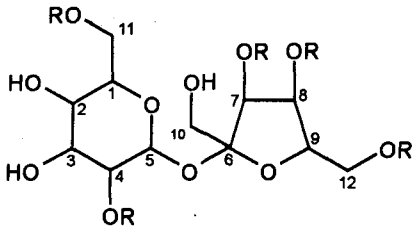
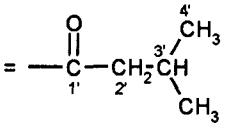
equivalent to 50-100 times the sample amount. Final purification was made on a YMC pack-sil column (250 mm × 10 mm) with HP 1100 series (Waldbronn, Germany) HPLC system. Mass spectra were obtained with an AutoSpec mass spectrometer (Micromass, Manchester, UK) equipped with an electrospray ionization (ESI) source. NMR spectra were obtained with a Bruker DMX-600 FT NMR spectrometer operating at 600 MHz and 150.9 MHz for ^{1}H and ^{13}C, respectively, with chloroform-*d* solvent as an internal solvent. For all experiments the temperature was stabilized at 298 K. Typical acquisition conditions for 2D-experiments (with the standard Bruker softwares: COSY,[9-10] HMQC,[11] and HMBC[12]) were 256 t_1 increments with 16-64 scans, 1024 t_2 points, and pulse repetition delays of 1.5-2 s.

The plant material used was purchased in Seoul, Korea. The crude seeds (2 kg) of *E. Lathyris* were extracted with CH_3OH three times at room temperature. The concentrated CH_3OH extract (820 g) was added to water and then partitioned successively with cyclohexane and acetonitrile. The acetonitrile layer (40 g), which showed a significant skin irritant activity, was subjected to column chromatography over silica gel with chloroform-methanol stepwise eluting mixtures (100:0 → 0:100), yielding fractions I, II, and III. Fraction I was fractionated into three compounds (2, 3, and 4) using silica gel column chromatography with various eluting mixtures of n-hexane-AcOEt (20:1 → 1:1). Compounds 2 (4.2 g), 3 (0.11 g), and 4 (1.2 g) were further purified by repeated HPLC with eluting mixtures of n-hexane-AcOEt (15:1), n-hexane-AcOEt (10:1), and n-hexane-AcOEt (8:1), respectively. Fraction III was fractionated into compounds 1 and 5 using silica gel column chromatography with eluting mixtures of chloroform-methanol (100:1 → 10:1). 1 (0.5 g) and 5 (0.05g) were further purified by repeated HPLC with eluting mixtures of chloroform-methanol (80:1) and chloroform-methanol (40:1), respectively (show the following Scheme 1).

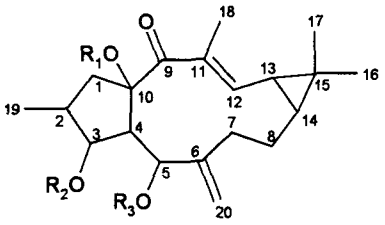


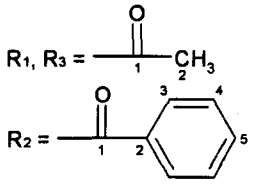
Scheme 1. Isolation scheme for sucrose ester (**1**), Euphorbia Factors L_1(**4**), L_2(**3**), L_3(**2**), and L_4(**5**) from the seeds of E. Lathyris



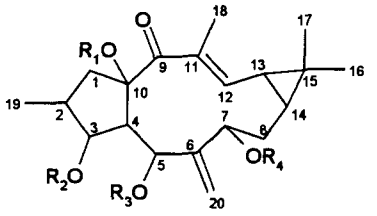


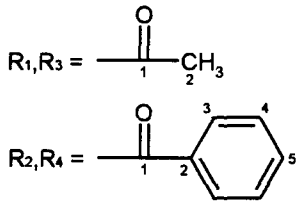
1 ; C37H62O16(762u)



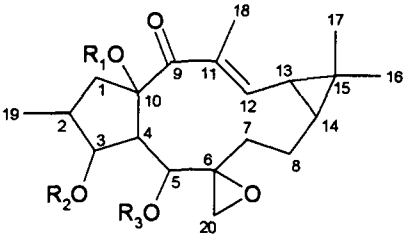


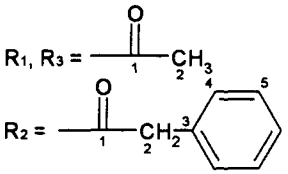
2 ; C31H38O7(522u)



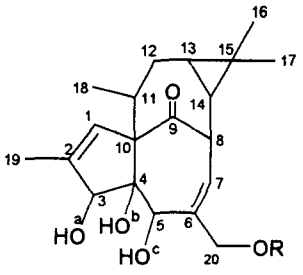


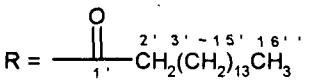
3 ; C38H42O9(642u)





4 ; C32H40O8(552u)





5 ; C36H58O6(586u)

Fig. 1. Structure of sucrose ester (**1**) and Euphorbia Factors L_1(**4**), L_2(**3**), L_3(**2**), and L_4(**5**)

RESULTS AND DICUSSION

The structure of compound **1** was characterized by NMR spectroscopy and the results are given in the Table 1. The assignments of the NMR spectra (^{1}H and ^{13}C) were confirmed by the 2D experiments such as COSY, HMQC, and HMBC. Some molecular formulae were confirmed by the high resolution ESI mass spectrometry. The ^{1}H NMR spectrum of the novel compound 1 exhibited signals for five-methylene (δ 2.22), five-methine (δ 2.07), and ten-methyl (δ 0.93) group protons. These signals corresponded to protons of isobutyl group ($(CH_3)_2$-CH-CH_2-), and it has been verified by the ^{1}H-^{1}H COSY experiment. The ^{13}C-NMR and DEPT spectra showed to have ten-methyl, eight-methylene, thirteen-methine, and six-quaternary carbons (one tertiary alcohol and five ester carbonyls). The ^{1}H-NMR and HMQC spectra exhibited additionally signals for eight-methine protons and three-methylene protons on carbons bearing oxygen atoms. The interpretation of all these spectra of compound 1 has revealed the presence of three structural elements; -CH_2-CH-CH-CH-CH-CH (glucose moiety), -CH-CH-CH-CH_2- (fructose moiety), and $(CH_3)_2$-CH-CH_2- (isovaleryl). Their connectivities were established from the long-range ^{1}H-^{13}C correlations observed in the HMBC spectrum (see Fig. 2 and Table 1). The positions of the five isovalerate ester groups could also be unambiguously determined by the HMBC experiment with evaluation of the ^{3}J(C,H) couplings between the oxymethine (H-4,7,8) and/or oxymethylene (H-11,12) protons and the ester carbonyl carbons (C-1') as shown in Figs. 2 and 3.

Hydrolysis of compound **1** (with 0.1 M NaOH in MeOH for 10 min at 50°C) gave the sucrose ester and isovalerate-removed sucrose, which were identified by the mass spectrometric method. In the mass spectrum of the partially hydrolyzed compound **1**, the peaks at m/z 785 ($[M+Na]^+$) and 365 ($[M-(COC_4H_8)_5+Na]^+$) confirmed the existence of the isovalerates.

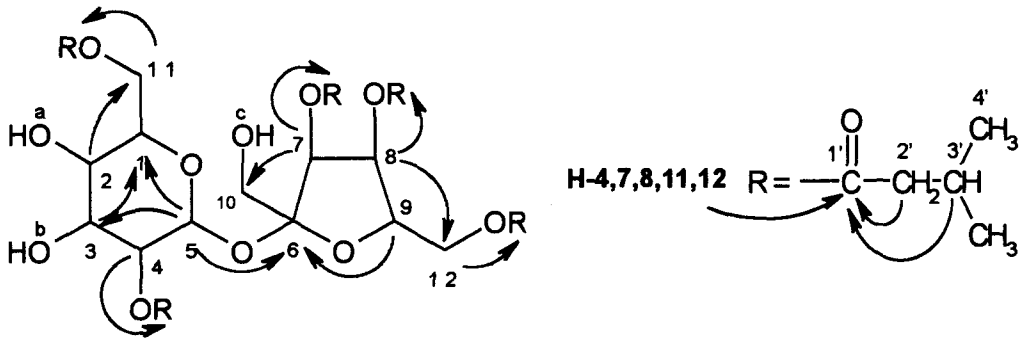


Fig. 2. Long-range ^{1}H-^{13}C correlations shown in the HMBC experiment of compound **1**

The complete assignments of the NMR spectra of the three diterpenes, compounds **2~4**, showed that these diterpenes are well known Euphorbia Factors L_3, L_2, and L_1, respectively.[4] The high resolution ESI mass spectrum of compound **5** indicated the molecular formula $C_{36}H_{58}O_6$ (Euphorbia Factor L_4) which were expected by the NMR experiments. Hydrolysis of this compound gave the ingenol, which was identified by the MS experiment. For the mass spectrometric observation, we produced the partial hydrolysis reaction products of compound **5** and found the hydrolyzed compound (348 u) and unhydrolyzed one (586 u). The mass difference (238 u) confirmed the existence of the hexadecanoate group on compound **5**

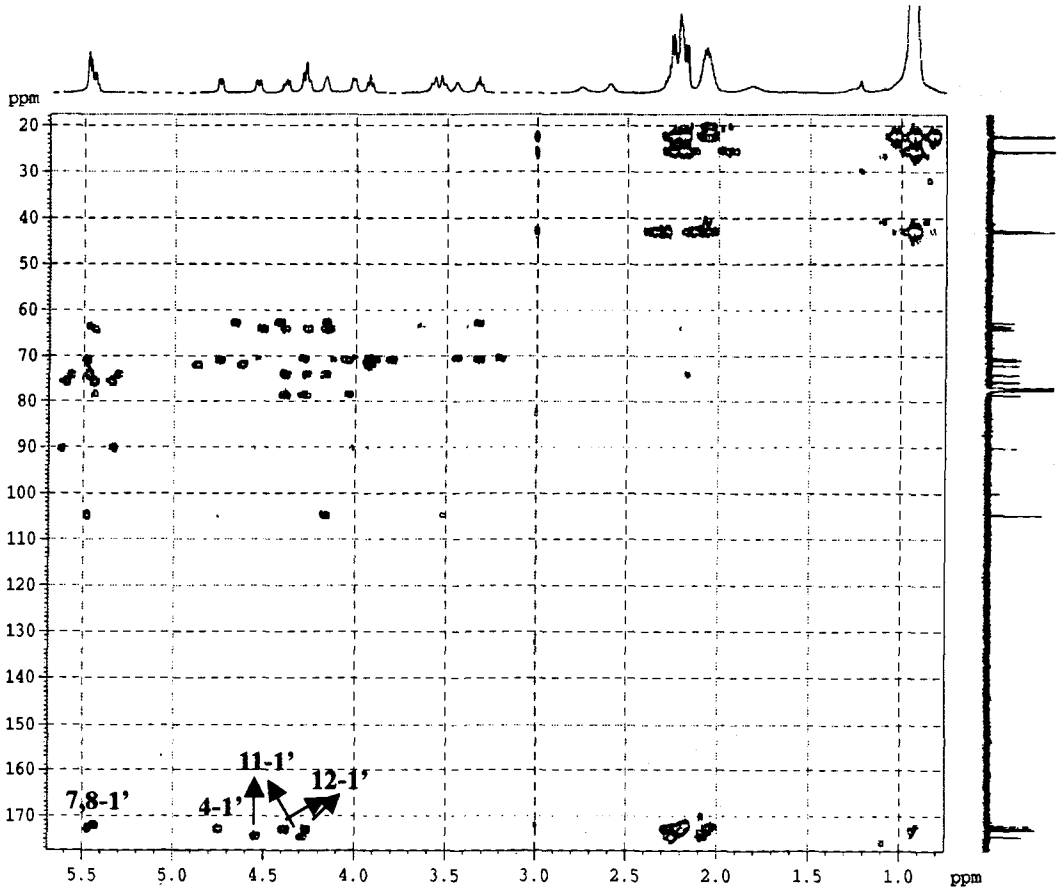


Fig. 3. HMBC spectrum of compound **1**. The mark on the cross peak shows a long range correlation between the corresponding proton and carbon

Table 1. ^{1}H, ^{13}C-NMR, COSY and HMBC data for compound 1

	Atom	DEPT	^{13}C (ppm)	^{1}H (ppm)	COSY	HMBC (H→C,10Hz)
Glucose moiety	1	CH	70.7	4.01	H-2, 11	C-2, 5
	2	CH	70.4	3.32	H-1, 3	C-1, 3, 11
	3	CH	70.9	3.93	H-2, 4	C-1, 2, 4
	4	CH	71.9	4.75	H-3, 5	C-3, 6, 1'
	5	CH	90.2	5.48	H-4	C-1, 3, 4, 6
	11	CH_2	62.7	4.28, 4.54	H-1	C-1, 2, 1'
Fructose moiety	6	Q	104.7			
	7	CH	75.5	5.46	H-8	C-6, 8, 10, 1'
	8	CH	74.0	5.45	H-7, 9	C-7, 9, 12, 1'
	9	CH	78.5	4.17	H-8, 12	C-6, 8, 12
	10	CH_2	63.6	3.55		C-6
	12	CH_2	64.1	4.28, 4.39	H-9	C-9, 9, 1'
Isovaleryl	1'	Q × 5	171.9~174.3			
	2'	CH_2 × 5	42.7~43.0	2.17~2.29	H-3'	C-1', 3', 4'
	3'	CH × 5	25.5~25.6	2.05~2.09	H-2', 4'	C-1', 2', 4'
	4'	CH_3× 10	22.2~22.3	0.9~1.0	H-3'	C-1', 2', 3'

Sucrose 4,7,8,11,12-pentaisovalerate (1): colorless amorphous power; HRESIMS m/z 762.4051 (calcd for $C_{37}H_{62}O_{16}$, 762.4038); ^{1}H- and ^{13}C-NMR data, see Table 1.

3-Benzoate-5,10-diacetate-lathyrol (Euphorbia Factor L_3) (2): white power; HRESIMS m/z 522.2640 (calcd for $C_{31}H_{38}O_7$, 522.2618); δ_H : 0.94(3H, H-19), 1.16(1H, H-14), 1.17(6H, H-16 and 17), 1.41(1H, H-13), 1.70(1H, H-1α), 1.72(3H, H-18), 1.75(1H, H-8α), 1.83(3H, H-$R_3$2), 1.94(1H, H-8β), 2.06(1H, H-7α), 2.10(1H, H-7β), 2.21(3H, H-$R_1$2), 2.35(1H, H-2), 2.90(1H, H-4), 3.52(1H, H-1β), 4.78(1H, H-20α), 5.01(1H, H-20β), 5.82(1H, H-3), 6.21(1H, H-5), 6.54(1H, H-12), 7.45(2H, H-$R_2$4), 7.57(1H, H-$R_2$5), 8.03(2H, H-$R_2$3).

3,7-Dibenzoate-5,10-diacetate-7-hydroxy-lathyrol (Euphorbia Factor L_2) (3): colorless amorphous powder, ESIMS m/z 642; δ_H : 0.88(3H, H-19), 1.21(6H, H-16 and 17), 1.22(3H, H-$R_3$2), 1.30(1H, H-14), 1.46(1H, H-13), 1.71(1H,, H-1α), 1.75(3H, H-18), 2.17(1H, H-8α), 2.17(3H, H-$R_1$2), 2.30(1H, H-8β), 2.30(1H, H-2), 2.90(1H, H-4), 3.36(1H, H-1β), 5.18(1H, H-20α), 5.48(1H, H-7), 5.50(1H, H-20β), 5.72(1H, H-3), 6.34(1H, H-5), 6.48(1H, H-12), 7.29(2H, H-$R_4$4), 7.40(2H, H-$R_2$4), 7.42(1H, H-$R_4$5), 7.52(1H, H-$R_2$5), 7.88(2H, H-$R_4$3), 8.01(2H, H-$R_2$3).

3-Phenyl-acetate-5,10-diacetate-6,20-epoxy-lathyrol (Euphorbia Factor L_1) (4): colorless amorphous powder, ESIMS m/z 552; δ_H : 0.62(3H, H-19), 0.89(1H, H-7α), 1.05(1H, H-

14), 1.16(3H, H-16), 1.17(3H, H-17), 1.31(1H,, H-1α), 1.44(1H, H-13), 1.68(1H, H-8α), 1.80(3H, H-18), 1.83(1H, H-4), 1.97(3H, H-$R_3$2), 2.04(1H, H-2), 2.08(1H, H-8β), 2.09(3H, H-$R_1$2), 2.10(1H, H-7β), 2.26(1H, H-20α), 2.45(1H, H-20β), 3.28(1H, H-1β), 3.54(2H, H-$R_2$2), 5.44(1H, H-3), 6.20(1H, H-5), 6.56(1H, H-12), 7.22(1H, H-$R_2$6), 7.23(2H, H-$R_2$4), 7.26(2H, H-$R_2$5).

Ingenol-20-palmitate (Euphorbia Factor L_4) (5): white syrup, HRESIMS m/z 586.4246 (calcd for $C_{36}H_{58}O_6$, 586.4233); δ_H : 0.68(1H, H-13), 0.85(3H, H-16'), 0.95(1H, H-14), 0.95(3H, H-18), 1.04(3H, H-16 or 17), 1.09(3H, H-16 or 17), 1.23(2H×12, H-4'~15'), 1.57(2H, H-3'), 1.75(1H, H-12α), 1.83(3H, H-19), 2.27(1H, H-12β), 2.28(2H, H-2'), 2.29(1H, H-11), 3.63(1H, H-5), 4.07(1H, H-8), 4.40(1H, H-3), 4.50(1H, H-20α), 4.69(1H,H-20β), 5.92(1H, H-1), 6.06(1H, H-7). ; δ_C : 14.1(CH_3, C-16'), 15.4(CH_3, C-16 or 17), 15.4(CH_3, C-19), 17.3(CH_3, C-18), 22.7(CH_2, C-15'), 23.0(CH, C-14), 23.1(CH, C-13), 23.9(Q, C-15), 24.9(CH_2, C-3'), 28.5(CH_3, C-16 or 17), 29.7~29.1(CH_2, C-4'~C-13'), 31.0(CH_2, C-12), 31.9(CH_2, C-14'), 34.3(CH_2, C-2'), 39.8(CH, C-11), 44.1(CH, C-8), 66.3(CH_2, C-20), 72.5(Q, C-10), 73.8(CH, C-5), 80.6(CH, C-3), 84.3(Q, C-4), 128.4(CH, C-7), 130.0(CH, C-1), 136.8(Q, C-6), 138.7(Q, C-2), 174.0(Q, C-1'), 206.6(Q, C-9).

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