A Novel *ent*-Kaurane Diterpenoid from the *Croton tonkinensis* GAGNEP

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A novel *ent*-kaurane diterpenoid, *ent*-1α-acetoxy-7β,14α-dihydroxy-kaur-16-en-15-on has been isolated from leaves of *Croton tonkinensis* GAGNEP. Its structure was determined by a combination of spectroscopy, X-ray crystallographic analysis and the chemical reaction acetylation.

Key words *Croton tonkinensis*; *ent*-kaurane; diterpenoid

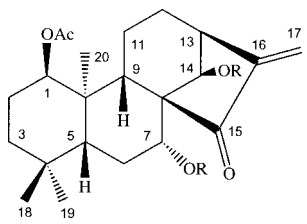
Croton tonkinensis GAGNEP belonging to Euphobiaceae grows wildly in Vietnam, and the leaves of this plant are prescribed for stomachache.[1)] The crude drug from the leaves was previously shown to have an inhibitory effect on malaria parasites.[2)] Later, Phan *et al.*[3)] reported the isolation and structural elucidation of a new *ent*-kaurane diterpenoid, *ent*-7β-hydroxy-15-oxokaur-16-en-18-yl acetate from the leaves. In the course of our investigation of the biologically active compounds from Vietnamese medicinal plants, we reinvestigated the chemical constituents of the leaves of *C. tonkinensis* and isolated six alkaloids.[4)] Recently, we collected a large amount of the leaves and isolated a novel *ent*-kaurane-type diterpenoid (**1**). This paper describes the isolation and structural characterization of compound **1**.

Compound **1** was obtained as white needles, $[\alpha]_D^{20}$ −96.0° (c=0.80, CH_3OH). The molecular formula was determined to be $C_{22}H_{32}O_5$ by high resolution electron impact (HR-EI)-MS. Its IR and UV spectra indicated the presence of a hydroxyl group (3272 cm^{-1}), a double bond (1651 cm^{-1}), an acetyl group (1727, 1243 cm^{-1}),[3)] and an absorption maximum at 230 nm, respectively. The ^{1}H-NMR spectrum of **1** (Table 1) showed the presence of three methyl groups, two *exo*-methylene protons and two hydroxyl groups. The ^{13}C-NMR spectrum of **1** (Table 1) showed resonances for 22 carbons including one conjugated ketone (δ 207.1), an acetyl group (δ 21.0, 169.7), a methine (δ 72.3) bearing an acetoxyl group, an *exo*-methylene (δ 116.8, 148.3), five methylenes and three quaternary carbons. The NMR spectral data of compound **1** were similar to those of *ent*-1α,11α-diacetoxy-7β,14α-dihydroxy-kaur-16-en-15-on (rastronol A),[5)] suggesting that compound **1** was a kaurane-type diterpenoid. The position of an acetyl group was determined at C-1 due to a long-range correlation between this group and H-1 in the heteronuclear multiple bond connectivity (HMBC) spectrum.[6)] Further, two hydroxyl groups were determined at C-7 and C-14, respectively, by HMBC correlation between 7-OH and C-7 as well as the 14-OH and C-13, C-14 and C-15 in the HMBC spectrum. The stereochemistry of **1** was determined by nuclear Overhauser effect spectroscopy (NOESY) spectrum (Table 1), in which the NOEs were observed between (1) H-5 and H-9, H-18; (2) H-7 and H-5, H-9; (3) H-1 and H-19, H-20; (4) H-14 and H-13, H-20 indicating that H-5, H-7, H-9, H-18, 1-OAc and 14-OH were *axial*, and H-1, H-13, H-19, H-20 and 7-OH were *equatorial*.

Acetylation of **1** with acetic anhydride in pyridine yielded a triacetate (**2**) with the molecular formula of $C_{26}H_{36}O_7$ by HR-EI-MS. The IR spectrum of **2** was similar to that of **1** except for the absence of absorption of the hydroxyl group. The

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data for Compound **1**

Position	^{1}H (δ)	^{13}C (δ)	HMBC	NOESY
1	4.68 m	72.3 d	H-2, H-20	H-20
2	1.96 m	22.3 t		H-1, H-20
	1.44 m			
3	1.38 dt (4.1, 14.0)	34.7 t	H-2, H-18, H-19	H-2, H-5, H-19
	1.16 m			
4		32.6 s	H-2, H-18, H-19	
5	1.24 dd (1.7, 12.4)	46.8 d	H-3, H-6	H-7, H-9
6	1.84 m	28.6 t	H-5	H-7, H-18
	1.68 m			
7	4.00 td (4.9, 12.4)	72.9 d	H-5, 7-OH	H-5, H-9, 14-OH
8		60.0 s	H-7, H-9, 7-OH	
9	1.51 d (8.8)	46.3 d	H-1, H-11	H-5, H-7
10		42.1 s	H-5, H-9, H-20	
11	1.28 dd (5.8, 16.4)	16.6 t	H-9, H-12	H-9
	1.21 m			
12	1.90 m	30.6 t	H-9, H-11, H-14	H-13, H-14
	1.61 m			
13	2.91 s	45.7 d	H-12, H-14, 14-OH	H-12, H-14
14	4.73 s	74.4 d	H-9, H-13, 14-OH	H-13, H-20
15		207.1 s	H-9, H-14, H-17	
16		148.3 s	H-14, H-17	
17	5.92 br s	116.8 t		
	5.38 br s			
18	0.90 s	33.1 q	H-19	H-5, H-6
19	0.83 s	21.3 q	H-18	H-3, H-20
20	1.06 s	18.0 q	H-1, H-5	H-1, H-19, H-14
1-OAc	1.92 s	169.7 s	H-1, 1-OAc	
		21.0 q		
7-OH	6.04 d (4.9)			H-20
14-OH	6.16 br s			



1: R = H

2: R = Ac

Fig. 1. The Structure of Compounds **1** and **2**

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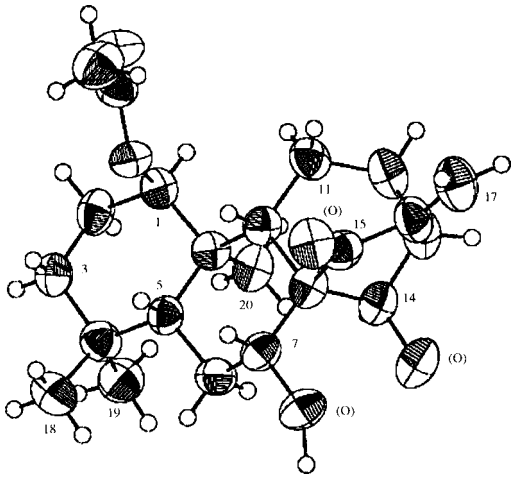

Fig. 2. ORTEP Drawing of **1**

^{1}H-NMR spectra of **2** also resembled those of **1**, but the signals of three acetyl groups (1.95, 2.01, 2.04) were observed confirming that compound **2** was a diacetate of compound **1**.

To confirm the relative structure of compound **1**, X-ray crystallographic analysis was carried out and an ORTEP drawing (Fig. 2) obtained.

Thus, compound **1** was determined to be *ent*-1α-acetoxy-7β,14α-dihydroxy-kaur-16-en-15-on. The absolute configuration of **1** remained to be clarified, even though we measured the circular dichroism (CD) spectrum which showed a first positive (369 nm: $\Delta\varepsilon$ +0.07), a second negative (330 nm: $\Delta\varepsilon$ −0.12), and a third negative Cotton effect (234 nm: $\Delta\varepsilon$ −2.57). However, *ent*-kaurane type was assigned to this structure since the present plant leaves produced the *ent*-kaurane series.[3]

Experimental

UV spectra were obtained on a Shimadzu UV-1610PC in MeOH. CD spectra were measured on a JASCO J-725 spectrometer in MeOH. IR spectra were measured on a JASCO FT/IR-5300 spectrophotometer. Specific optical rotation was measured on a JASCO DIP-1000 polarimeter with CH_3OH as solvent. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Unity 600 (600 MHz for ^{1}H-NMR and 150 MHz for ^{13}C-NMR), using DMSO-d_6 and $CDCl_3$ as solvents. Chemical shifts were given with TMS (δ 0.00) being used as internal standard (^{1}H-NMR), and δ 39.7 (ppm) from DMSO-d_6 and 77.03 (ppm) from $CDCl_3$ as standards (^{13}C-NMR). Mass spectra were recorded on a JEOL JMS AX-500 spectrometer. X-ray reflection data were measured on a DIP image diffractometer using Mo $K\alpha$ radiation (λ= 0.71073Å). Column chromatography was carried out on silica gel 60 (Merck).

Plant Material *Croton tonkinensis* Gagnep was collected in Socson, Hanoi, Vietnam in October 1997 and identified by Dr. Nguyen Kim Dao (Institute of Ecology and Biological Resources, Hanoi, Vietnam). The voucher specimen (No 43) was deposited at the Herbarium of the Institute of Ecology and Biological Resources, National Centre of Natural Sciences and Technology of Vietnam.

Extraction and Isolation Dried leaves of *C. tonkinensis* (1000 g) were extracted with MeOH. The filtrate was concentrated under reduced pressure, followed by acidification using HCl (5%) until pH=4—5. The solution was shaken with light-petrol in order to eliminate carotenoids and alkalized by NH_4OH until pH=9—10, then extracted with $CHCl_3$, and evaporated to give the crude extract (2.50 g) which was subjected to repeated silica gel column chromatography using *n*-hexane–EtOAc gradient to afford compound **1** (25.2 mg) as white needles.

Compound **1**: White needles (from acetone); mp 97—98 °C; $[\alpha]_D^{20}$ −96.0° (c=0.80, CH_3OH). UV λ_{max} (MeOH) (log ε): 230 (2.66). CD (MeOH) λ_{ext} nm ($\Delta\varepsilon$) 369 (+0.07), 330 (−0.12), 234 (−2.57); IR (KBr) cm^{-1}: 3272, 1727, 1651, 1464, 1373, 1243, 1178, 1154, 1118, 1095, 1028, 994, 961. ^{1}H- and ^{13}C-NMR (DMSO): see Table 1. HR-MS (EI) *m/z*: 376.2231 (M^+, Calcd for $C_{22}H_{32}O_5$: 376.2250).

Acetylation of 1 Compound **1** (2 mg) in pyridine (1 ml) was acetylated with acetic anhydride (1 ml) and workup as usual afforded triacetate (**2**) (1.4 mg). $[\alpha]_D^{20}$ −25.6° (c=0.50, CH_3OH). UV λ_{max} (MeOH) (log ε): 233 (3.55). IR (KBr) cm^{-1}: 2951, 1735, 1648, 1467, 1368, 1238, 1058, 1031, 975. ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, s, 19-H), 0.94 (3H, s, 18-H), 1.23 (1H, m, 3-H), 1.28 (1H, m, 11-H), 1.31 (3H, s, 20-H), 1.48 (1H, m, 3-H), 1.53 (1H, m, 11-H), 1.58 (1H, dd, J=2.8, 12.1 Hz, 5-H), 1.62 (1H, m, 2-H), 1.82 (1H, m, 12-H), 1.84 (1H, br d, J=12.4 Hz, 6-H), 1.88 (1H, m, 6-H), 1.92 (1H, br d, J=8.5 Hz, 9-H), 1.95 (3H, s, 1-$\underline{CH_3}$CO), 1.98 (1H, m, 2-H), 2.01 (3H, s, 7-$\underline{CH_3}$CO), 2.04 (3H, s, 14-$\underline{CH_3}$CO), 2.12 (1H, m, 12-H), 3.05 (1H, br s, 13-H), 4.87 (1H, br s, H-1), 5.30 (1H, dd, J=4.4, 12.4 Hz, 7-H), 5.40 (1H, s, 17-H), 6.04 (1H, br s, 14-H), 6.14 (1H, s, 17-H). ^{13}C-NMR ($CDCl_3$) δ: 16.5 (C-11), 18.6 (C-20), 21.2 (C-19), 21.4 (1, 7, 14-$\underline{CH_3}$CO), 22.6 (C-2), 24.3 (C-6), 32.5 (C-12), 33.0 (C-4), 33.1 (C-18), 34.9 (C-3), 43.0 (C-10), 44.2 (C-13), 47.3 (C-5), 48.5 (C-9), 60.9 (C-8), 72.8 (C-1), 73.8 (C-7), 74.9 (C-14), 117.8 (C-17), 145.6 (C-16), 169.6 (1-$CH_3\underline{CO}$), 170.3 (7-$CH_3\underline{CO}$), 170.8 (14-$CH_3\underline{CO}$), 204.2 (C-15). HR-MS (EI) *m/z*: 460.2470 (M^+, Calcd for $C_{26}H_{36}O_7$: 460.2461).

Crystal data for **1**. Data collection: DIP Image plate. Cell refinement: Scalepack (HKL). Data reduction: maXus.[7] Program used to solve structure: maXus.[7] Refinement: on F^2 full matrix least-squares. Diffractometer: DIP Image plate. $C_{22}H_{32}O_5$, MW 376.2, orthorhombic, $P2_12_12_1$, a=7.0240 (6)Å, b=12.121 (3)Å, c=24.823 (2)Å, α=90.00°, β=90.00°, γ=90.00°, V=2113.4 (6)Å^3, Z=4, Mo $K\alpha$ radiation, λ=0.71073, μ=0.080 mm^{-1}, 3627 reflections, 253 parameters; only coordinates of H atoms refined, R=0.0893, R_w=0.2894, S=1.087.

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