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(*all-E*)-*β*-Carotene and (*all-E*)-*β,ε*-carotene 3,3'-diol from *Toona Sureni*

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ABSTRACT
Two carotenoids have been isolated from the leaves of *Toona sureni* (Blume) Merr. The structures of the compounds were determined to be (*all-E*)-*β*-carotene (**1**) and (*all-E*)-*β,ε*-carotene 3,3'-diol (**2**), based on UV-vis, FTIR, NMR and EIMS spectra.

Keywords: *Toona sureni*, carotenoid, *β* -carotene and *β,ε*-carotene 3,3'-diol

1. INTRODUCTION
The plant of *Toona sureni* (Blume) Merr belongs to the Meliaceae family, and in Indonesia it is found in Sumatra, Java and Sulawesi. Various parts of the tree, especially the bark and root, are used for medicinal purposes, e.g. to treat diarrhoea, while the leaves extracts have been reported to have an antibiotic effect. The bark and fruits have been used for production of essential oils.[1]

Carotenoids are among the most widespread and important natural pigments. Together with chlorophylls they are found in all organisms which involve in photosynthesis.[2] Their biological activities are as a vitamin A precursor,[2] antioxidant,[4-7]anticancer,[8-10] antivirus,[11]and cytotoxic against cultured human colon tumor cells.[12] Literature search revealed that a number of different compounds have previously been isolated from the leaves of the plant, including tetranortriterpenoid (surenin, surenone and surenolactone).[13,14] Another species of *Toona* genus, e.g. *Toona ciliata* contains limonoid[15], terpenoid[16] and the essential oil from the leaves.[17] In this paper, we report the isolation and structural elucidation of two carotenoids, (*all-E*)-*β*-carotene (**1**) and (*all-E*)-*β,ε*-carotene 3,3'-diol (**2**), from the leaves of the title plant.

2. EXPERIMENTAL

2.1 *General Experimental Procedures*
NMR spectra were recorded on a JEOL JNM ECA- 500 NMR spectrometer (^{1}H NMR 500 MHz and ^{13}C NMR 125 MHz). Chemical shifts were referenced to acetone-d_6 (δ_H 2.05 and δ_C 29.9 and 206.7). IR spectrum was recorded on a JASCO FT-IR 460 plus spectrophotometer in KBr pellet. UV-vis spectrum was recorded on SECOMAM UV S S100 spectrophotometer in methanol solution. EIMS (70 eV) was recorded on Funnigan MAT SSQ 710 spectrometer.

2.2 *Plant material*
Plant materials were collected in Padang, West Sumatera, Indonesia in July 2007, and identified by the staff of the Herbarium of the Andalas University (ANDA), Padang, and the voucher specimen (M. Taufik Ekaprasada, 0107, ANDA.Fr) was deposted in the herbarium.

2.3 *Extraction and isolation*
The finely chopped fresh leaves (5 kg) of the plant were macerated with MeOH (20 L) for 5 days and the process was repeated twice. The combined extracts were evaporated under reduced pressure to a small volume (ca. 1 L). The MeOH extract was saponified with 5 % KOH-MeOH for 12 h at room temperature. Then unsaponifiable matter was extracted with Et_2O and then with EtOAc and water added until two layers were formed. The organic layers were red due the presence of carotenoids and aqueous layer was green due to the presence of chlorophylls. The ether layer was separated and washed with H_2O and dried over anhydrous Na_2SO_4 and then evaporated to dryness. The residue (28 g) was subjected to a column chromatography of silica gel and eluted with an increasing percentage of Me_2CO in *n*-hexane (100: 0; 90:10; 80: 20; 75: 25; 70: 30 and 60: 40 v/v) togive 4 fractions. After evaporating the solvens,fractions I was crystallized from methanol to give compound 1 (20 mg), red needles meeting out at 172-173 ºC, the other 3 fractions were not investigated due to give broadening spots on t.l.c The acetate layer was separated and washed with water, dried over anhydrous Na_2SO4 and evaporatedto dryness. The residuwas subjected to a column chromatography of silica the residue gel and eluted with an increasing percentage of Me_2CO in *n*-hexane (100: 0; 90:10; 80: 20; 75: 25; 70: 30 and 60: 40 v/v).Fractions with the same R_f on TLC were combined and rechromatographed on the silica gel column and eluted with *n*-hexane-Me_2CO (8:2 v/v) and was further recrystalized from *n*-hexane to give a red solid of compopund **2** (10 mg).

Compound **1** was obtained as a red needles (methanol), m. p. 172-173 ^oC; UV (MeOH) λ_{max}: 275, 428 and 448 nm, and 476 nm; IR (KBr) ν_{max}: 2915 (C-H), 1624 (C=C) olefinic conjugated, 1445 (C-H) methylene), 1371 (C-H) of methyl group, and 976 (C-H) olefinic cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6): see Table 1;^{13}C NMR (125 MHz,

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acetone-d_6): see Table 1;EIMS (70 eV) m/z 536 $[M]^+$ (100), 444 (5), 430 (1), 307 (7).

Compound 2 was obtained as a red powder (*n*-hexane), m. p. 166-168 ^oC; UV (MeOH) λ_{max}: 420, 443 and 471 nm; IR (KBr) ν_{max}: 3426 (OH), 2920 (C-H), 1635 (C=C) cm^{-1}; ^1H NMR (500 MHz, acetone-d_6): see Table 2; ^{13}C NMR (125 MHz, acetone-d_6): see Table 2; EIMS (70 eV) m/z 568.4 $[M]^+$ (18), 550.4 (100), 476.3 (6), 458.3 (36), 429.3 (5), 337.3 (10).

3. RESULTS DISCUSSION

Uv-vis spectrum of compound **1** gave absorpsion maxima at 428 and 448 nm, and 476 nm. The absence of near-UV (about 330 nm) absorbance was characteristic of the *all*-E arrangement of the double bonds.

Infra red spectrum showed absorption at wavelength of 2915, 1624, 1445, 1371, and 967 cm^{-1}. The peaks did not show any characteristic of hydroxyl, amine, carbonyl or acetylenic groups. Based on spectrum it was predicted that compound was hydrocarbon. The presence of single peaks at 967 cm^{-1} indicated that compound was trans isomer, since trans isomer would give double peak at 967 cm^{-1}.

Tabel-1: NMR data of compound **1** in acetone-d_6

No	δ_H (*mult.*, *J* in Hz	δ_C	DEPT[a]
1,1’	-	34.28	C
2,2’	1.46 (*m*)[b]	39.66	CH_2
3,3’	1.62 (*m*)[b]	19.27	CH_2
4,4’	2.02(*t*, 6.01)	33.12	CH_2
5,5’	-	129.39	C
6,6’	-	137.92	C
7,7’	6.14 (*d*, 7.3)	126.66	CH
8,8’	6.14 (*d*, 7.3)	138.43	CH
9,9’	-	136.02	C
10,10’	6.12 (*d*, 10.8)	130.84	CH
11,11’	6.65 (*dd*, 10.8; 14.9)	125.04	CH
12,12’	6.35 (*d*, 14.9)	137.23	CH
13,13’		136.48	C
14,14’	6.25 (*d*, 10.8)	132.42	CH
15,15’	6.63 (d, 10.8)	129.99	CH
16,16’	1.03 (*s*)	28.98	CH_3
17,17’	1.03 (*s*)	28.98	CH_3
18,18’	1.72 (*s*)	21.76	CH_3
19,19’	1.97 (*s*)	12.82	CH_3
20,20’	1.98 (*s*)	12.77	CH_3

[a]assignments are based on DEPT; [b]coupling constant could not be determined due to overlapping multiples.

The ^1H NMR spectrum of compound **1**showed peaks at δ 6.65 ppm (dd, J_1= 10.8 and J_2=14.9), 6.63 ppm (d, J = 10.8), 6.35 ppm (d, J = 14.9), 6.25 ppm (J = 10.8), 2.02 ppm (t, J = 6.01), 1.97 ppm (s), 1.72 ppm (s), 1.62 ppm (m), 1.46 ppm (m), 1.03 (s). The ^1H NMR spectrum of compound **1**showed the presence 56 of protons. H_{11} proton gave doublet-doublet peat at 6.65 ppm, since it was coupled by H_{10} and H_{12} proton (1H;dd;J = 10.8 Hz and J_2 = 14.9 Hz).Doublet peak at 6.63 ppm was assumed to be H_{15} peak, since it was coupled by H_{14} proton (1H;d; J =10.8 Hz). Doblet peak at 6.35 ppm was assumed to be H_{12} which was coupled by H_{11} proton (1H; d; J = 14.9 Hz). H_7 and H_8 protons were coupled each other and gave doublet peak at 6.14 ppm (2H; d; J = 7.3 Hz). Doublet peak at 6.12 ppm was thought to be H_{10} proton which was coupled by H_{11} proton (1H; d; J = 10.8 Hz).

Triplet peak at 2.02 ppm indicated two protons of C_4 which were coupled by protons of C_3 (2H; t; J = 6.01 Hz). Singlet peak at 1.97 ppm indicated 6 protos of C_{19} and C_{20} (6H; s; CH_3 of C_{19}; CH_3 of C_{18}; CH_3 of C_{20}). Single peak at 1.72 ppm indicated 3 protons of C_{18} (3H; s; CH_3 of C_{18}). Multiplet peak at 1.60 ppm indicated 2 protons of C_3 which were coupled by two protons of C_2 and two protons of C_4 (2H; m; CH_2 of C_3). Multiplet peak at 1.46 ppm indicated two protons of C_2 which were coupled by two protons of C_3 (2H; m; CH_2 of C_2). Singlet peak at 1.03 ppm indicated six protons of C_{16} and C_{17} (6H; s; CH_3 of C_{16} andC_{17}).

The mass spectrum showed that moleculer ion was 536. This moleculer ion corresponds to $C_{40}H_{56}$. Double bond equivalence (DBE) was 13. This DBE showed the presence of 11 doublet bonds and two rings the fragmentations showed loss toluene (M-92) and xylene (M-106), the characteristic of carotenoid compounds.

The ^{13}C NMR showed the presence of 20 peaks. This indicated the presence of 20 carbon aatoms so that both spectra indicated that this molecule was symmetrical, that is why ^{13}C NMR gave peaks instead of 40 peaks.

The DEPT 90 ^{13}C NMR spectrum showed the presence of 7 tertiary carbon peaks. The DEPT 135 ^{13}C NMR spectrum showed the presence of 5 tertiary carbon peaks, 3 secondary carbon peaks. ^{13}C NMR spectrum showed the presence of 20 peaks, so that there were 5 quaternary carbon peaks. Since the molecule was symmetrical, there were

10 primary, 5 secondary, 14 tertiary, and 10 quaternary carbon atoms. Based on spectroscopic evidence compound **1** was (*all-E*)-*β*-carotene.

Compound **2** showed absorption maxima at 420, 443 and 471 nm, indicating that it has a carotenoid chromophore. The absence of near-UV (about 330 nm) absorbance was characteristic of the *all*-E arrangement of the double bonds. The FTIR spectrum confirmed a carbon-carbon double bond (ν_{maks}1635 cm^{-1}) and C-H stretching (ν_{maks} 2920 cm^{-1}), as well as revealed the presence of OH (ν_{maks} 3426 cm^{-1} broad). In the EIMS the compound showed a molecular ion peak at*m/z* 568.4 corresponds to the formula $C_{40}H_{55}O_2$. Further structural analysis was made by using extensive NMR data including ^{1}H and 13C NMR, ^{1}H-^{1}H COSY, HMQC and HMBC spectra.

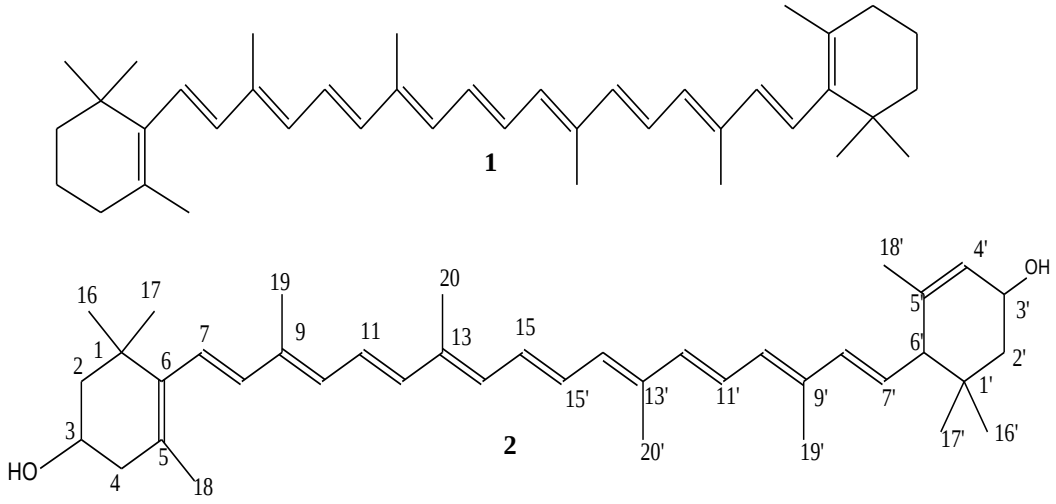

Fig-1: Structure of carotenoid compounds isolated from *Toona sureni* (Blume) Merr

The ^{1}H NMR spectrum of **2** (Table) showed a crowded methyl and olefinic signals at δ_H 0.97 – 1.98 (10 methyl signals) and 5.51 – 7.4 ppm (15 methine signals), respectively. The ^{1}H NMR spectrum also showed two broad signals at δ_H 3.90 and 4.17 ppm attributed to two secondary hydroxyl groups, and three methylene signal in the region 1.34 – 2.30 ppm. These proton chemical shift assignments were made through interpretation of the ^{1}H-^{1}H COSY spectrum which showed connectivities between proton signals within each isolated spin system.

Tabel-2: NMR data of compound **2** in acetone-d_6[a]

No	δ_H (*mult.*, *J* in Hz	COSY	δ_C	DEPT	HMBC (H ⇔ C
1	-	-	37.60	C	-
2	1.41 (*dd*, 11.6,11.6 1.76 (*m*)[b]	H-3	49.57	CH_2	C-1, C-3, C-4, C-16
3	3.9 (*br m*)	H-2	64.45	CH	-
4	2.03(*m*)[b] 2.30 (*dd*, 16.5, 5.5)	- -	43.59	CH_2	C-2, C-3, C-5
5	-	-	127.64	C	-
6	-	-	138.43	C	-
7	6.18 (*d*, 15.3)	H-8	126.69	CH	C-6, C-9, C-19
8	6.20 (*d*, 15.3)	H-7	131.78	CH	C-9, C-10
9	-	-	136.37	C	-
10	6.18 (*d*, 15.3)	H-8	139.20	CH	C-9, C-19
11	6.73 (*m*)[b]	H-8, H-10, H-12, H-14	131.21	CH	C-12, C-13
12	6.33 (*m*)[b]	10,14	133.60	CH	C-11, C-14, C-20
13	-	-	137.28	C	-
14	6.41 (*d*, 13.4)	H-12, H-15	138.50	CH	C-12, C-20
15	6.73 (*m*)[b]	H-8, H-10, H-12, H-14, H-15’	125.97	CH	C-12, C-13
16	1.05 (*s*)	-	29.10	CH_3	C-1, C-2, C-6, C-17
17	1.05 (*s*)	-	29.10	CH_3	C-1, C-2, C-6, C-16
18	1.72 (*s*)	-	21.97	CH_3	C-4, C-5, C-6
19	1.92 (*s*)	-	13.19	CH_3	C-8, C-9, C-10
20	1.98 (*s*)	-	12.89	CH_3	C-11, C-12, C-13
1’	-	-	34.73	C	-

2’	1.34 (*dd*, 12.8,7.3) 1.79 (*m*)[b]	H-3’	45.77	CH_2	C-1’, C-3’, C-4’, C-6’, C-16’, C-17’
3’	4.17 (*br m*)	H-2’	65.39	CH	-
4’	5.51 (*d*, 10.4)	H-6’	127.13	CH	C-5’, C-6’, C-18’
5’	-	-	135.96	C	-
6’	2.43 (*d*, 10.4)	H-4’	55.85	CH	C-5’, C-7’
7’	5.52 (*dd*, 15.0, 10.4)	H-8’	130.21	CH	C-5’, C-6’, C-18’
8’	6.22 (*d*, 15.0)	H-6’, H-7’	132.38	CH	C-6’, C-9’, C-10’, C-19’
9’	-	-	136.45	C	-
10’	6.18 (*d*, 15.3)	H-8’	139.28	CH	C-9’, C-19’
11’	6.74 (*m*)[b]	H-8’, H-10’ H-12’, H-14’	131.21	CH	C-13’, C-14’
12’	6.33 (*m*)[b]	H-10’, H-14’	133.66	CH	C-11’, C-14’, C-20’
13’	-	-	137.32	C	-
14’	6.41 (*d*, 13.4)	H-12’, H-15’	138.53	CH	C-12’, C-20’
15’	6.74 (*m*)[b]	H-8’, H-10’, H-12’, H-14’	126.05	CH	C-12’, C-13’
16’	0.84 (*s*)	-	24.43	CH_3	C-1’, C-2’, C-6’, C-17’
17’	0.97 (*s*)	-	30.10	CH_3	C-1’, C-2’, C-6’,C-16’,
18’	1.59 (*s*)	-	23.11	CH_3	C-4’, C-5, C-6’
19’	1.98 (*s*)	-	12.89	CH_3	C-11’, C-12’, C-13’
20’	1.98 (*s*)	-	12.82	CH_3	C-11’, C-12’, C-13’

[a]assignments are based on DEPT, HMQC, and HMBC; [b]coupling constant could not be determined due to overlapping multiplets

The ^{13}C NMR spectrum of compound **2** revealed signals consistent with the structure and was assigned through the interpretation of the HMQC and HMBC spectra, as shown in the Table 2. From these analysis and by comparison with those reported from the literature.[18-21], compound **2** was assigned as (*all-E*)-*β,ε*-carotene 3,3’-diol. The stereochemistry of the secondary hydroxyl was not determined, however the configuration of the double bond was made by using the coupling constant of the vinyl signals.

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