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Studies on the Constituents of the Roots of *Cassia torosa*. II.[1] The Structures of Two Dimeric Tetrahydroanthracenes

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Two new dimeric tetrahydroanthracene derivatives, torosaols I (1) and II (2), were isolated from the fresh roots of *Cassia torosa* CAV. along with pinselin, questin, 7-methyltorosachrysone, singueanol-I, germichrysone, germitorosone, methylgermitorosone, and phlegmacins A_2 and B_2. The structures of compounds 1 and 2 were established as 3,3′,4,4′-tetrahydro-2,3,3′,8,8′,9,9′-heptahydroxy-6,6′-dimethoxy-3,3′,7,7′-tetramethyl-10,10′-bi-1(2*H*)-anthracenone and 5-[3′,4′-dihydro-3′,8′,9′-trihydroxy-6′-methoxy-3′,7′-dimethyl-1′(2′*H*)-anthracenon-10′-yl]-3,4-dihydro-9,10-dihydroxy-3-hydroxymethyl-7-methoxy-3,8-dimethyl-1*H*-naphtho[2,3-*c*]pyran-1-one, respectively, on the basis of spectral evidence.

Compounds 1 and 2 showed cell growth inhibitory activity against the KB cells.

Keywords *Cassia torosa*; Leguminosae; dimeric tetrahydroanthracene; bitetrahydroanthracene; torosaol I; torosaol II; cytotoxic agent; bianthracenone

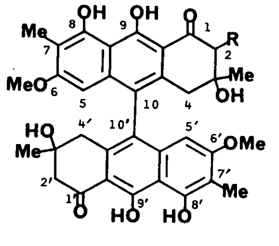
In a previous paper,[2] we reported the isolation of two new naphthalenic lactones, 7-methyltoralactone and 7-methyltoralactone 10-methyl ether, from the fresh roots of *Cassia torosa* CAV.

In this paper, we report the isolation, structural determination, and cytotoxic activity against cultured KB cells of two new dimeric tetrahydroanthracene derivatives, torosaols I (**1**) and II (**2**), which have been obtained, along with pinselin (**3**),[3] questin (**4**),[4] 7-methyltorosachrysone (**5**),[5] singueanol-I (**6**),[3,6] germichrysone (**7**),[7] germitorosone (**8**),[8] methylgermitorosone (**9**),[8] and phlegmacins A_2 (**10**) and B_2 (**11**),[9,10] from the fresh roots of this plant.

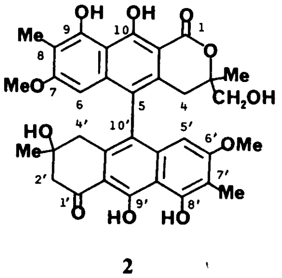
Torosaol I (**1**), yellowish-brown prisms, mp 300 °C (dec.), gave a yellowish-green coloration by the $FeCl_3$ reaction. The high-resolution mass spectrum (MS) of **1** gave the molecular formula $C_{34}H_{34}O_{11}$ (m/z 618.2111 as the base peak), and major fragment ions were observed at m/z 600 ($M^+ - H_2O$) and 582 ($M^+ - 2H_2O$). Characteristic absorptions at 281, 308, 322, 334 and 407 nm in the ultraviolet (UV) spectrum and at 3400, 2900, 1620, 1600 and 1500 cm^{-1} in the infrared (IR) spectrum suggested a 3,4-dihydro-1(2*H*)-anthracenone homologue such as **5**, and therefore **1** was assumed to be a dimeric 3,4-dihydro-1(2*H*)-anthracenone derivative.[3,4] The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** (Table I) showed the presence of two aliphatic methyl groups (δ 1.29, 1.32), two aromatic methyls (δ 2.16, 6H), two geminally coupled methylenes [δ 2.47, 2.58 (each d, $J = 16.3$ Hz); 2.53, 2.65 (each d, $J = 17.2$ Hz)], a non-coupled methylene (δ 2.83), two methoxyls (δ 3.49, 3.50) and a methine (δ 4.31), together with two aromatic protons (δ 6.03, 6.04) and four chelated hydroxyls (δ 9.93, 10.29, 15.13, 16.70). Thus **1** was indicated to be a heterodimer of two different 3,4-dihydro-1(2*H*)-anthracenes. A comparison of the ^{1}H-NMR chemical shifts of **1** and **6** showed that **1** had one more methine proton (δ 4.31) and one less methylene group (δ 2.81) than **6**, so that **1** was considered to be a dimeric compound composed of **5** and **8**. The assignment of functional groups in the cyclohexanone rings was performed on the basis of the nuclear Overhauser effect (NOE). In the different NOE experiments, irradiations of two aliphatic methyl protons at δ 1.29 and 1.32 produced signals at δ 2.47, 2.58, 2.83 and δ 2.53, 2.65, 4.31, and their signals were assigned as shown in Table I. A comparison of the ^{1}H-NMR chemical shifts of **5** and **8** indicated that the C_3-Me (δ 1.32), $C_{3'}$-Me (δ 1.29), $C_{5,5'}$-H (δ 6.03, 6.04), $C_{6,6'}$-OMe (δ 3.49, 3.50) signals in **1** had upfield shifts owing to an anisotropic effect of the twisted naphthalene rings, while the signals of C_{10}-H in **5** and **8** had disappeared. Thus, the binding sites of their units in **1** were between each C-10 of germitorosone (**8**) and 7-methyltorosachrysone (**5**).

Therefore, the plane structure of **1**, which we have named torosaol I, was shown to be 3,3′,4,4′-tetrahydro-2,3,3′,8,8′,9,9′-heptahydroxy-6,6′-dimethoxy-3,3′,7,7′-tetramethyl-10,10′-bi-1(2*H*)-anthracenone.

Torosaol II (**2**), appearing as yellowish-brown prisms, mp 230 °C (dec.), gave a green coloration by the $FeCl_3$ reaction. The high-resolution MS gave the molecular formula $C_{34}H_{34}O_{11}$ (m/z 618.2068 as the base peak), and major fragment ions were obtained at m/z 600 ($M^+ - H_2O$), 582 ($M^+ - 2H_2O$), 569 ($M^+ - H_2O - OMe$) and 554 ($M^+ - 2H_2O - CO$). The UV and IR spectra were similar to



1: R = OH
6: R = H



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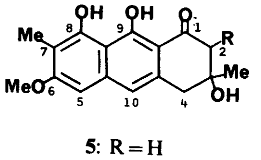

5: R = H
8: R = Me

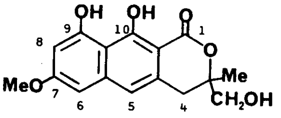

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Chart 1

TABLE I. ^{1}H-NMR Data for Compounds **1**, **2**, **5**, **8** and **12** (δ Values in $CDCl_3$)

	1[a]	**8**[b]	**5**[b]		**2**[a]	**12**[b]
C_2-H	4.31 s	4.28 d (J=1.5 Hz)	2.84 br s	C_3-Me	1.26 s	1.41 s
C_2-OH		3.84 d (J=1.5 Hz)		C_3-CH_2-OH	3.44 d (J=12.2 Hz)	3.66 d (J=12.2 Hz)
C_3-Me	1.32 s	1.53 s	1.43 s		3.71 d (J=12.2 Hz)	3.84 d (J=12.2 Hz)
C_4-H	2.53 d (J=17.2 Hz)	3.11 br s	3.04 br s	C_4-H	2.18 d (J=16.3 Hz)	2.71 d (J=16.6 Hz)
	2.65 d (J=17.2 Hz)				3.04 d (J=16.3 Hz)	3.44 d (J=16.6 Hz)
C_{10}-H	—	6.89 t (J=1.0 Hz)	6.89 t-like	C_5-H	—	6.93 br s
C_5-H	6.03 s[c]	6.53 s	6.53 s	C_6-H	5.98 s	6.57 d (J=2.4 Hz)
C_6-OMe	3.49 s[d]	3.90 s	3.92 s	C_7-OMe	3.49 s[d]	3.89 s
C_7-Me	2.16 s	2.17 s	2.18 s	C_8-Me	2.19 s	
				C_8-H		6.54 d (J=2.4 Hz)
C_8-OH	9.93 s	9.58 d (J=1.0 Hz)	9.97 d (J=1.0 Hz)	C_9-OH	9.91 d (J=0.9 Hz)	9.54 d (J=2.4 Hz)
C_9-OH	15.13 s	14.61 d (J=1.0 Hz)	16.13 d (J=1.0 Hz)	C_{10}-OH	14.13 d (J=0.9 Hz)	13.70 s
$C_{2'}$-H	2.83 br s			$C_{2'}$-H	2.82 br s	
$C_{3'}$-Me	1.29 s			$C_{3'}$-Me	1.28 s	
$C_{4'}$-H	2.47 br d (J=16.3 Hz)			$C_{4'}$-H	2.35 br d (J=16.3 Hz)	
	2.58 d (J=16.3 Hz)				2.57 d (J=16.3 Hz)	
$C_{10'}$-H	—			$C_{10'}$-H	—	
$C_{5'}$-H	6.04 s[c]			$C_{5'}$-H	6.07 s[c]	
$C_{6'}$-OMe	3.50 s[d]			$C_{6'}$-OMe	3.50 s[d]	
$C_{7'}$-Me	2.16 s			$C_{7'}$-Me	2.17 s	
$C_{8'}$-OH	10.29 s			$C_{8'}$-OH	10.34 d (J=0.9 Hz)	
$C_{9'}$-OH	16.70 s			$C_{10'}$-OH	16.70 d (J=0.9 Hz)	

a) Measured at 400 MHz. *b*) Measured at 100 MHz. *c*, *d*) Assignments in any column may be reversed.

those of 3,4-dihydro-1(2*H*)-anthracene compounds, showing maxima at 270, 317, 330sh, 372sh, 390, 418sh nm and at 3400, 2950, 1625, 1600 and 1510 cm^{-1}. The ^{1}H-NMR spectrum of **2** showed the presence of two aliphatic methyl (δ 1.26, 1.28), two aromatic methyl (δ 2.17, 2.19), three geminally coupled methylene [δ 2.18, 2.57 (each d, J= 16.3 Hz), δ 2.35, 3.04 (each d, J=16.3 Hz), δ 3.44, 3.71 (each d, J=12.2 Hz)], a non-coupled methylene (δ 2.82), two methoxyl (δ 3.49, 3.50), two aromatic (δ 5.98, 6.07) protons, and four chelated hydroxyl groups (δ 9.91, 10.34, 14.13, 16.70). From the MS and ^{1}H-NMR data, **2** was considered to be a structural isomer of **1**. A spin-decoupling experiment using **2** showed that irradiation of two aliphatic methyl protons at δ 1.26 and 1.28 sharpened the signals at δ 3.44, 3.04 and at δ 2.82, 2.35, 2.57. Thus, one unit in **2** was shown to have a 7-methyltorosachrysone structure, and the other to have one more geminally coupled oxymethylene (δ 3.44, 3.71) and one less methine (δ 1.32) group compared with the ^{1}H-NMR data for **1**, whereas a chelated hydroxyl (δ 14.13) of **2** appeared more upfield than in **1**.

This latter unit is considered to be a 3,4-dihydronaphtho[2,3-*c*]pyran-1-one skeleton like that of cassialactone (**12**)[11] obtained from *Cassia obtusifolia*. Because a comparison of the ^{1}H-NMR data of the corresponding protons in 3,4-dihydronaphtho[2,3-*c*]pyran-1-one unit with **12** showed that the unit had one more aromatic methyl (δ 2.19) and two less aromatic protons (δ 6.54, 6.93) than **12**, so that the structure of the 3,4-dihydro-1*H*-naphtho[2,3-*c*]pyran-1-one skeleton was cassialactone containing a methyl group at the naphthalene ring. A comparison of the ^{1}H-NMR data for the methylcassialactone unit in **2** with **12** indicated that the C_3-Me [δ 1.26 (0.15 ppm)], C_3-oxymethylene [δ 3.44 (0.22 ppm), 3.71 (0.13 ppm)], C_4-methylene [δ 2.18 (0.53 ppm), 3.04 (0.40 ppm)], C_6-H [δ 5.98 (0.41 ppm)] and C_7-OMe [δ3.49 (0.39 ppm)] signals for methylcassialactone in **2** showed an upfield shift owing to an anisotropic effect of the twisted naphthalene rings. By comparison of the ^{1}H-NMR spectra of 7-methyltorosachrysone and **12**, the binding site was considered to be located between the C-10 of the 7-methyltorosachrysone moiety and the C-5 of the cassialactone moiety combined with the methyl group at C-8.

Thus, the plane structure of **2** was shown to be 5-[3′,4′-dihydro-3′,8′,9′-trihydroxy-6′-methoxy-3′,7′-dimethyl-1′(2*H*)-anthracenone-10′-yl]-3,4-dihydro-9,10-dihydroxy-3-hydroxymethyl-7-methoxy-3,8-dimethyl-1*H*-naphtho[2,3-*c*]pyran-1-one.

The effective doses for 50% inhibition (ED_{50}) of the *in vitro* KB cell line by torosaol I (**1**) and torosaol II (**2**) were 1.7 and 5.5 μg/ml, respectively. Details of the cytotoxicity of these tetrahydroanthracene compounds will be published elsewhere.

Experimental

All the melting points were taken on a Yanagimoto micro-melting-point apparatus and are uncorrected. The UV spectra were obtained on a Hitachi 200-10 spectrophotometer, and the IR spectra on a JASCO IR A-2 spectrophotometer. The NMR spectra were taken on JEOL JNM-FX-100 and JNM-GX-400 instruments. The MS were obtained on a Hitachi RMU-7M spectrometer. Column chromatography was performed on silicic acid (SiO_2) (Mallinckrodt), and preparative thin layer chromatography (PLC) was conducted on Silica gel 60 (Merck) treated with 0.5 N oxalic acid.

Isolation The column chromatography were the same as described in our previous paper.[2] Fraction 4 gave crude **7** (665 mg) with crystallization from C_6H_6, and the mother liquid was rechromatographed with C_6H_6–AcOEt (19:1→9:1) to afford **3** (13 mg), **4** (10 mg), **5** (4 mg), **6** (60 mg), and **7** (102 mg). Fraction 5 gave **8** (16 mg), **9** (1 mg), **1** (24 mg) and **2** (4 mg) on PLC with C_6H_6–AcOEt (4:1). Fraction 6 with C_6H_6–AcOEt (1:1) gave **10** (1 mg) and **11** (3 mg) on PLC with C_6H_6–AcOEt (3:2).

Torosaol I (1) Crude crystals were recrystallized from C_6H_6 to give yellowish brown prisms; mp 300 °C (dec.). UV $\lambda_{max}^{dioxane}$ nm (log ε): 235 (4.67), 281 (4.89), 308 (4.05), 322 (4.17), 334 (4.05), 407 (4.29). IR ν_{max}^{KBr} cm^{-1}: 3400, 2900, 1620, 1600, 1500. MS m/z: 618 (M^+, 100%), 600 ($M^+ - H_2O$, 9), 582 ($M^+ - 2H_2O$, 11). High-resolution MS m/z: Calcd for $C_{34}H_{34}O_{11}$: 618.2099. Found: 618.2118. The ^{1}H-NMR data are shown in Table I.

Torosaol II (2) Crude crystals were recrystallized from C_6H_6 to give yellowish brown prisms; mp 230 °C (dec.). UV $\lambda_{max}^{dioxane}$ nm (log ε): 232 (4.56), 270 (4.86), 317 (4.03), 330sh (3.91), 372sh (4.12), 390 (4.19), 418sh (3.91). IR ν_{max}^{KBr} cm^{-1}: 3400, 2950, 1625, 1600, 1510. MS m/z: 618 (M^+, 100%), 600 ($M^+ - H_2O$, 36), 582 ($M^+ - H_2O - OMe$, 18), 554 ($M^+ - 2H_2O - CO$, 17). High-resolution MS m/z: Calcd for $C_{34}H_{34}O_{11}$: 618.2099. Found: 618.2068. The ^{1}H-NMR data are shown in Table I.

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References and Notes

1) Part XXVII in series "Studies on the Constituents of Purgative Crude Drugs". For Part XXVI, see S. Kitanaka, K. Ogata and M. Takido, *Phytochemistry*, **29**, 999 (1990).
2) S. Kitanaka and M. Takido, *Chem. Pharm. Bull.*, **33**, 4912 (1985).
3) S. Kitanaka and M. Takido, *Chem. Pharm. Bull.*, **37**, 511 (1989).
4) S. Kitanaka and M. Takido, *Chem. Pharm. Bull.*, **32**, 860 (1984).
5) S. Kitanaka, H. Igarashi, and M. Takido, *Chem. Pharm. Bull.*, **33**, 971 (1985).
6) M. Endo and H. Naoki, *Tetrahedron*, **35**, 2449 (1980).
7) S. Takahashi, M. Takido, U. Sankawa, and S. Shibata, *Phytochemistry*, **15**, 1295 (1976).
8) M. Takido, S. Kitanaka, S. Takahashi, and T. Tanaka, *Phytochemistry*, **21**, 425 (1982).
9) S. Takahashi, S. Kitanaka, M. Takido, U. Sankawa, and S. Shibata, *Phytochemistry*, **16**, 999 (1977).
10) S. Kitanaka and M. Takido, *Phytochemistry*, **21**, 2103 (1982).
11) S. Kitanaka and M. Takido, *Phytochemistry*, **20**, 1951 (1981).