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Bitetrahydroanthracenes from Flowers of *Cassia torosa* CAV.[1)]

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A new bitetrahydroanthracene derivative, torosaol-III, was isolated from the flowers of *Cassia torosa* CAV. along with physcion, 5,7′-physcionanthrone-physcion, 5,7′-biphyscion, torosanin-9,10-quinone, 5,7-dihydroxychromone, naringenin, and chrysoeriol. The structure of torosaol-III was established as 3,3′,4,4′-tetrahydro-3,3′,8,8′,9,9′-hexahydroxy-6,6′-dimethoxy-3,3′-dimethyl-1(2*H*),1′(2*H*)-5,7′-bianthracenone on the basis of spectral and chemical evidence. Dimeric tetrahydroanthracenes exhibited cytotoxic activity against KB cells in the tissue culture.

Keywords *Cassia torosa*; Leguminosae; tetrahydroanthracene; bitetrahydroanthracene; torosaol-III; cytotoxic effect

As part of the isolation of bioactive constituents and new natural products, we have been screening extracts from the *Cassia* genus (Leguminosae) for cytotoxic properties.

In our previous papers,[2−12)] with regard to *Cassia torosa*, we reported the isolation of anthraquinones, anthrones, hydroanthracenes, naphthalenic lactones, and flavonoids, including cytotoxic principles, torasaols I and II (dimeric anthracene) and torosaflavone C (a flavonoid having two five-member rings), from the ripe and unripe seeds, seedlings, roots, and leaves of this plant. In this paper, we wish to report the isolation and structure elucidation of a new dimeric anthracene derivative, torosaol-III, and the cytotoxic activity of dimeric hydroanthracenes against cultured KB cells.

A benzene extract from the whole of the floral parts of *C. torosa* was chromatographed to afford compounds **1**—**8** as described in Experimental.

Compound **2**, $C_{32}H_{24}O_9$, and compound **3**, $C_{32}H_{22}O_{10}$, were estimated as 5,7′-physcionanthrone-physcion (**2**) and 5,7′-biphyscion (**3**), respectively, from the ^{1}H-NMR spectra and the nuclear Overhauser effect (NOE) experiments. Recently, compounds of the same plane structure, 5,7′-biphyscion (floribundone-1) and 5,7′-physcionanthrone-physcion (floribundone-2), were reported to be components of the leaves of *Cassia floribunda* CAV.[13)] However, the circular dichroism (CD) spectra of **3** showed opposite Cotton effects to those of floribundone-1, suggesting that **3** is an atropisomer of floribundone-1 caused by restricted rotation around the biphenyl linkage. Compound **2** was converted into **3** by treatment with a basic condition. Therefore, in combination with the evidence given for **8** (see below), compounds **2** and **3** were elucidated as atropisomers of floribundone-2 and -1, respectively, and the names, (−)-floribundone-2 and (−)-floribundone-1, are proposed.

Compound **8**, named torosaol-III, was obtained as yellow needles, mp 205—207 °C, $[\alpha]_D$ −311.0°. The high resolution mass spectrum (HR-MS) of **8** gave the molecular formula $C_{32}H_{30}O_{10}$ [*m*/*z* 574.1840 (M^+)], and major fragment ions were observed at *m*/*z* 556 (M^+-H_2O), 538 (M^+-2H_2O) (base peak), and 269 [$(M^+-2H_2O)/2$]. Characteristic absorptions at 278, 317 sh and 401 nm in the ultraviolet (UV) spectrum and at 3600—3200, 1625, and 1580 cm^{-1} in the infrared (IR) spectrum suggested a dimeric 3,4-dihydro-1(2*H*)-anthracenone structure such as phlegmacins.[7)] ^{1}H-NMR spectrum of **8** showed the presence of two aliphatic methyl groups (δ 1.36, 1.48), four methylenes (δ 2.75, 2.80 (4H), 2.81, 2.93), two methoxy groups (δ 3.77, 3.81), as well as four non-coupled aromatic protons (δ 6.46, 6.68, 6.71, 6.98), and four chelated hydroxyls (δ 9.91, 10.21, 16.16, 16.39) (Table

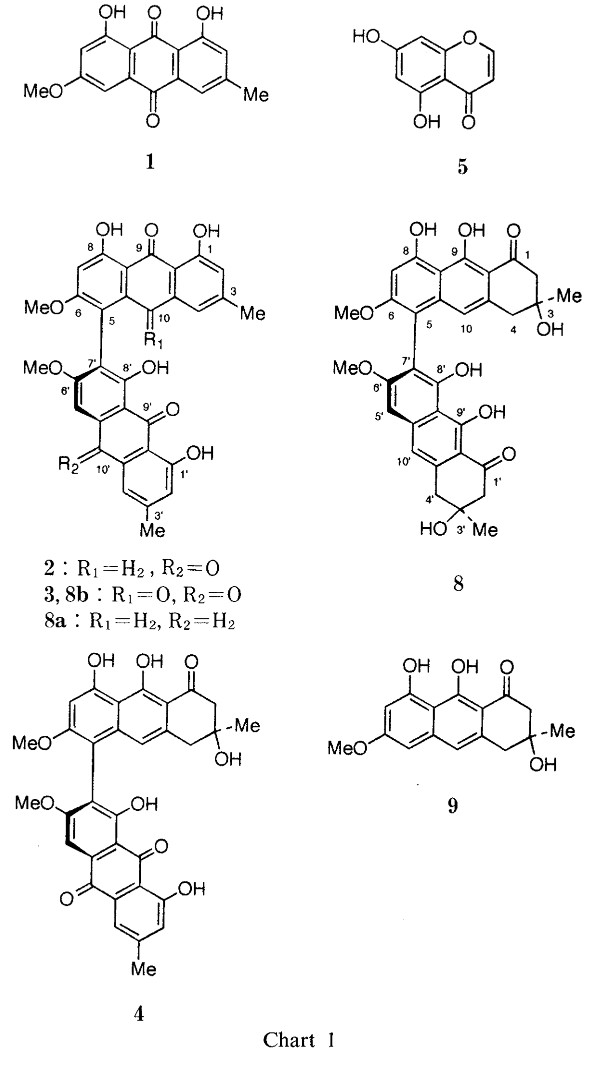

Chart 1

TABLE I. ^{1}H- and ^{13}C-NMR Data$^{a)}$ for **8**

Carbon No.	H	C	Carbon No.	H	C
1	—	200.9	1′		201.8
2	2.75 d, J=16.8 Hz 2.80 d, J=16.8 Hz		2′	2.81 d, J=12.0 Hz 2.93 d, J=12.0 Hz	51.0
3	—	71.0	3′	—	71.1
4	2.86 s	43.4	4′	3.07 d, J=16.1 Hz 3.12 d, J=16.1 Hz	43.4
4a	—	134.5	4a′	—	135.3
10	6.46 s	115.6	10′	6.98 s	117.7
10a	—	139.0	10a′	—	140.6
5	—	107.7$^{b)}$	5′	6.68 s	98.2
6	—	161.5	6′	—	
7	6.71 s	97.8	7′	—	109.6
8	(10.21 s)	160.7	8′	(9.91 s)	156.8
8a	—	107.8$^{b)}$	8a′	—	108.2
9	(16.39 s)	167.7	9′	(16.16 s)	166.1
9a	—	108.4	9a′	—	108.3
3-Me	1.36 s	28.9	3′-Me	1.48 s	29.1
6-OMe	3.81 s	56.2	6′-OMe	3.77 s	55.9

a) Measured in $CDCl_3$ at 400 MHz, with tetramethyl silane (TMS) as the internal standard. The following abbreviations are used: s, singlet; d, doublet. *b)* May be interchanged.

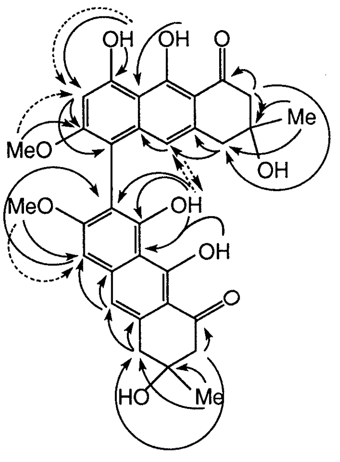

Fig. 1. NOEs and ^{1}H–^{13}C Long-Range Correlations Observed for **8**
HMBC ——→, NOE - - -→.

I). Thus, **8** was estimated to be a dimeric compound of torosachrysone (**9**).[3] Comparisons of the ^{1}H-NMR chemical shifts of **8** and **9** indicated that the H-10 (δ 6.46) signal in **8** had shifted upfield by 0.41 ppm owing to an anisotropic effect of two twisted naphthalene rings, while doublet signals of H-5 or H-7 in **9** had disappeared in **8**. Thus, the plane structure of **8** was suggested to be a 5,7′-bitorosachrysone.

In the ^{13}C-NMR spectrum, 16 quarternary aromatic carbon signals were observed. The ^{1}H-detected multiple quantum coherence (HMQC)[14,15] spectra and the ^{1}H-detected heteronuclear multiple bond connectivity (HMBC)[15] were measured, and the linkage position of two units and the assignments were investigated through the detection of ^{1}H–^{13}C long-range correlation as shown in Fig. 1. In the HMBC spectrum, the quarternary aromatic carbon at δ 107.7 showed cross peaks with two protons at δ 6.71 (H-7) and 6.46 (H-10). On the other hand, the quarternary aromatic carbon at δ 109.6 exhibited long-range correlation with protons at δ 6.68 (H-5′) and 9.91 (OH-8′), so that the carbons at δ 107.7 (C-5) and at δ 109.6 (C-7′) must be connected to each other. This dimeric structure was supported by nuclear Overhauser effect differental spectroscopy (NOEDS), that is, the observation of NOE between the chelated hydroxyl proton at δ 9.91 (OH-8′) and the proton at δ 6.46 (H-10) indicated a 5,7′-biphenyl system. Therefore, the compound is represented by the formula **8**.

Treatment of **8** with hydrochloric acid and acetic acid gave the dianhydro derivative, 5,7′-biphyscion-9-anthrone (**8a**), $[\alpha]_D$ −165.5°, which afforded, on flushing with oxygen gas in an alkaline solution, 5,7′-biphyscion (**8b**), $[\alpha]_D$ −246.2°, whose spectral data were identical with those of **3** excect for optical properties. Since the axis chirality of the 5—7′ position of **8b** should be the same as that of **8**, the stronger Cotton effect of **8b** than **3** suggests that the optical purity of **3** is lesser than that of **8b**.

The CD curve of **8** exhibited strong negative first (286 nm) and positive second (267 nm) Cotton effects due to the ^1B_b transitions of the two naphthalene chromophores, and this shows that the two long axes of the naphthalene nuclei in **8** are twisted in an anticlockwise manner, that is, S (Chart 1).[10,16−18] Configurations of the 3,3′-positions in two torosachrysone units are presumed to be the same as that of torosachrysone[17] in the seeds. Therefore, the structure of **8** was established as (3S,3′S)-3,3′,4,4′-tetrahydro-3,3′,8,8′,9,9′-hexahydroxy-6,6′-dimethoxy-3,3′-dimethyl-1(2H),1′(2H)-5,7′-(R)-bianthracenone.

The *in vitro* effective dose for 50% inhibition (ED_{50}) of torosaol-III (**8**) and tosanin-9,10-quinone (**4**) against the KB cell line was 1.8 and 2.4 μg/ml, respectively.

Experimental

All melting points were taken on a Yanagimoto micro-melting-point apparatus and are uncorrected. UV spectra were obtained on a Hitachi 200-10 spectrophotometer, and IR spectra were recorded on a JASCO IR A-2 spectrophotometer. NMR spectra were taken on a JEOL FX-100 or JEOL JNM GX-400 instrument; chemical shifts are given in ppm relative to internal tetramethylsilane (TMS). MS were obtained on a

Hitachi RMU-7M spectrometer. Column chromatography was performed on silicic acid (SiO_2) (Mallinckrodt) or Sephadex LH-20 (25—100 μm, Pharmacia Fine Chemical Co., Ltd.).

Extraction and Isolation Dried flowers (765 g) of *Cassia torosa* Cav. collected at the Drug Plant Garden of the College of Pharmacy, Nihon University, were extracted with C_6H_6 (16 l × 4) at room temperature. The extract was concentrated *in vacuo* to give a brown mass (15.3 g). The mass (15.2 g) was chromatographed on a SiO_2 column C_6H_6 to give fraction 1 , fractions 2—5 with C_6H_6–AcOEt (9 : 1), fraction 6 with C_6H_6–AcOEt (4 : 1), and fraction 7 with C_6H_6–AcOEt (1 : 1). Fraction 1 was rechromatographed to give physcion (**1**) (1 mg) and **2** (3.2 mg). Fraction 2 was rechromatographed on SiO_2 with hexane–AcOEt (9 : 1) to give **3** (1.4 mg). Fraction 3 was rechromatographed to afford torosanin-9,10-quinone (**4**) (1 mg). Fractions 4, 5 and 6 were chromatographed on Sephadex LH-20 and eluted with MeOH to give **5** (2.5 mg), naringenin (**6**) (4.9 mg), and chrysoeriol (**7**) (3.2 mg), respectively. Fraction 7 gave **8** (342 mg) on concentration and recrystallization from C_6H_6.

(−)-Floribundone-2 (2) Orange needles from C_6H_6, mp >300 °C. UV $\lambda_{max}^{dioxane}$ nm (log ε): 222 (4.66), 273 (4.45), 284 sh (4.41), 360 (4.17), 436 (4.03). CD ($c=0.0017$, dioxane) $\Delta\varepsilon^{22}$: 462 (−1.68), 424 (0), 405 (+0.49), 355 (0), 335 (−0.29), 323 (0), 295 (+2.96), 288 (0), 278 (−4.94), 263 (0), 252 (+2.86). IR ν_{max}^{KBr} cm^{-1}: 3500—3100, 1640, 1615, 1590, 1470, 1360, 1300, 1260, 1200, 1130, 1100, 1050, 940, 910, 820, 810. ^{1}H-NMR ($CDCl_3$) δ: 2.28 (3H, br s, 3-Me), 2.49 (3H, br s, 3′-Me), 3.85 (3H, s, OMe), 3.88 (2H, br s, H-10), 6.55 (2H, s, H-4, -7), 6.67 (1H, br s, H-2), 7.12 (1H, br s, H-2′), 7.56 (1H, s, H-5′), 7.69 (1H, d-like, H-4′), 12.08 (1H, s, OH-1′), 12.28 (1H, s OH-8′), 12.42 (1H, s, OH-1), 13.05 (1H, s, OH-8). MS *m*/*z*: 552 (M$^+$, 100%), 521 (M$^+$ −OMe, 9), 269 (M$^+$ −$C_{16}H_{11}O_5$, 9). HR-MS *m*/*z*: Calcd for $C_{32}H_{24}O_9$: 552.1419. Found: 552.1421.

(−)-Floribundone-1 (3) Orange needles from C_6H_6, mp >300 °C. UV $\lambda_{max}^{dioxane}$ nm (log ε): 222 (4.54), 255 sh (4.28), 280 (4.40), 438 (4.12). CD ($c=0.0020$, dioxane) $\Delta\varepsilon^{22}$: 464 (−2.98), 428 (0), 408 (+0.80), 370 (0), 338 (−0.44), 322 (0), 295 (+5.80), 288 (0), 277 (−9.30), 262 (0), 252 (+3.42). IR ν_{max}^{KBr} cm^{-1}: 3550—3350, 1670, 1620, 1600, 1550, 1500, 1460, 1420, 1380, 1365, 1310, 1230, 1190, 1170, 1120, 1030, 840, 830. ^{1}H-NMR ($CDCl_3$) δ: 2.36 (3H, br s, Me-3), 2.47 (3H, br s, Me-3′), 3.83 (3H, s, OMe), 3.89 (3H, s, OMe), 6.82 (1H, s, H-7), 7.04 (1H, d-like, H-2), 7.09 (1H, br s, H-2′), 7.41 (1H, br s, H-4), 7.55 (1H, br s, H-5′), 7.67 (1H, d-like, H-4′). MS *m*/*z*: 566 (M$^+$, 100%), 535 (M$^+$ −OMe, 94), 517 (M$^+$ −Me−H_2O, 13), 297 (M$^+$ −$C_{15}H_{19}O_5$, 53), 283 (M$^+$/2, 16). HR-MS *m*/*z*: Calcd for $C_{32}H_{22}O_{10}$: 566.1212. Found: 566.1214.

5,7-Dihydroxychromone (5) Pale yellow needles from MeOH, mp 182 °C. UV λ_{max}^{MeOH} nm: 208, 223 sh, 243, 255, 293. IR ν_{max}^{KBr} cm^{-1}: 3100—2900, 1640, 1610. ^{1}H-NMR (Me_2CO-d_6) δ: 6.22 (1H, d, $J=6.3$ Hz, H-3), 6.26 (1H, d, $J=2.4$ Hz, H-2), 6.39 (1H, d, $J=2.4$ Hz, H-8), 8.07 (1H, d, $J=6.3$ Hz, H-2), 9.77 (1H, br s, OH-7), 12.77 (1H, s, OH-5).

Torosaol-III (8) Yellow needles from C_6H_6, mp 205—207 °C, $[\alpha]_D^{24}$ −311.0° ($c=0.50$, $CDCl_3$). UV $\lambda_{max}^{dioxane}$ nm (log ε): 230 (4.39), 278 (4.72), 317 sh (3.40), 401 (4.20). CD ($c=0.00116$, dioxane) $\Delta\varepsilon^{22}$: 420 (−7.5), 347 (0), 340 (+1.7), 333 (0), 286 (−107.9), 278 (0), 267 (106.4), 232 (0). IR ν_{max}^{KBr} cm^{-1}: 3600—3200, 1625, 1580. MS *m*/*z*: 574 (M$^+$, 15%), 556 (M$^+$ −H_2O, 37), 538 (M$^+$ −2H_2O, 100), 507 (M$^+$ −2H_2O−OMe, 13), 269 [(M$^+$ −2H_2O)/2]. HR-MS: Calcd for $C_{32}H_{32}O_{10}$: 574.1837. Found: 574.1840. The ^{1}H- and ^{13}C-NMR data are shown in Table I.

(−)-5,7′-Biphyscion (8b) from Torosaol-III (8) Concentrated hydrochroric acid (0.07 ml) was added to a solution of torosaol-III (**8**) (24 mg) in acetic acid (2 ml). The mixture was left to stand for 1 h at 80 °C, diluted with H_2O, and extracted with AcOEt. Crystallization of the product from AcOEt gave (−)-5,7′-biphyscion-9-anthrone (**8a**) (16 mg) as pale yellow needles, mp 280 °C, $[\alpha]_D^{22}$ −165.5° ($CHCl_3$, $c=0.30$). UV $\lambda_{max}^{dioxane}$ nm (log ε): 224 (4.67), 260 (4.37), 274 (4.30), 306 (4.18), 359 (4.46). CD ($c=0.0010$, dioxane) $\Delta\varepsilon^{22}$: 410 (+1.5), 397 (0), 370 (−11.0), 341 (0), 344 (+0.4), 324 (0), 321 (−0.1), 295 (+4.0), 277 (0), 268 (−2.9), 247 (0), 242 (+2.2), 239 (0), 232 (−13.1), 226 (0), 216 (+23.7). IR ν_{max}^{KBr} cm^{-1}: 2920, 1614, 1594, 1558, 1485, 1463, 1369, 1249, 1203, 1180, 1128, 1090, 1056, 913, 807, 751. ^{1}H-NMR ($CDCl_3$) δ: 2.28 (3H, s, Me-3), 2.40 (3H, s, Me-3′), 3.80 (3H, s, OMe-8), 3.81 (1H, d, $J=23.5$ Hz, H-10), 3.82 (3H, s, OMe-8′), 4.03 (1H, d, $J=23.5$ Hz, H-10′), 6.55 (2H, s, H-4 and H-7), 6.59 (1H, s, H-5′), 6.66 (1H, br s, H-2), 6.73 (1H, br s, H-2′), 6.74 (1H, br s, H-4′), 12.29 (1H, s, OH-1′), 12.41 (1H, s, OH-1), 12.73 (1H, s, OH-8′), 13.02 (1H, s, OH-8). HR-MS: Calcd for $C_{32}H_{26}O_8$: 538.1625. Found: 538.1585.

Compound **8a** (12 mg) was coverted, on treatment (50 °C, 5 min) with 0.1 N sodium hydroxide (1 mg), into 5,7′-biphyscion (**8b**) (7 mg), mp >300 °C. $[\alpha]_D^{22}$ −246.2° ($c=0.31$, $CHCl_3$). CD ($c=0.002$, dioxane) $\Delta\varepsilon^{23}$: 466 (−12.8), 431 (0), 408 (+4.3), 370 (0), 338 (−1.8), 324 (0), 296 (+29.6), 288 (0), 278 (−35.5), 264 (0), 251 (+16.3). The IR and ^{1}H-NMR data were identical with those of **3**.

References and Notes

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