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Pauferrol A, a novel chalcone trimer with a cyclobutane ring from *Caesalpinia ferrea* mart exhibiting DNA topoisomerase II inhibition and apoptosis-inducing activity

Hiroshi Nozaki,[a,*] Ken-ichiro Hayashi,[a] Masahiro Kido,[b] Kazuyuki Kakumoto,[a] Shogo Ikeda,[a] Nobuyasu Matsuura,[c] Hiroyuki Tani,[b] Daisuke Takaoka,[d] Munekazu Iinuma[e] and Yukihiro Akao[f]

[a]*Department of Biochemistry, Faculty of Science, Okayama University of Science, 1-1 Ridai-cho, Okayama 700-0005, Japan*
[b]*Integrated Center for Sciences (INCS), Ehime University, Bunkyo-cho, Matsuyama 790-8577, Japan*
[c]*Department of Life Science, Faculty of Science, Okayama University of Science, 1-1 Ridai-cho, Okayama 700-0005, Japan*
[d]*Department of Chemistry, Faculty of Education, Ehime University, Bunkyo-cho, Matsuyama 790-8577, Japan*
[e]*Department of Pharmacognosy, Gifu Pharmaceutical University, 6-1 Mitahora-higashi 5-choume, Gifu 502-8585, Japan*
[f]*Gifu International Institute of Biotechnology, 1-1Naka-Fudogaoka, Kakamigahara, Gifu 504-0838, Japan*

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Abstract—Pauferrol A (**1**), a unique chalcone derivative was isolated from the stems of *Caesalpinia ferrea* Mart, and the structure was determined on the basis of 2D-NMR spectroscopy to be a chalcone trimer fused by a cyclobutane ring. This new chalcone trimer showed potent inhibitory activity against human topoisomerase II, with an IC_{50} value of 2.1 μM, and cell proliferation inhibitory activity through the induction of apoptosis in human leukemia HL60 cells, with an IC_{50} value of 5.2 μM. To our knowledge, this is the first report of the isolation and structure of this chalcone trimer and its biological activity.
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DNA topoisomerases (topo I and II) are found in all prokaryotic and eukaryotic cells, and in some viruses. They have been shown to participate in almost all cellular transactions of DNA, among which replication, transcription and genetic recombination are the most significant. DNA topoisomerases catalyze topological rearrangements of DNA, such as relaxation of supercoiled DNA, and catenation and decatenation of DNA rings. The cellular functions of topo II are involved in proliferative processes, such as DNA replication, chromosome condensation, and chromosome segregation. Therefore, topo II is regarded as an important clinical target for anti-cancer drugs.[1] In the continuous search for topo II inhibitors in plant extracts,[2,3] we have reported that nepalensinols, new stilbene oligomers from *Kobresia neparensis* (Cyperaceae), are potent topo II inhibitors[4,5] and we have shown their structure–activity correlation.[6] These facts prompted us to screen for more potent topo II inhibitors from plant extracts. Consequently, we found pauferrol A (**1**), a novel chalcone trimer fused with a cyclobutane ring, to be a potent topo II inhibitor from the stem of *Caesalpinia ferrea* Mart. Additionally, **1** exhibited potent apoptosis-inducible activity in the human acute myeloid leukemia HL 60 cell line, similar to clinically used anti-cancer drugs with topo II inhibitory activity.[7,8] Here, we describe the isolation, structural characterization, and biological activity of pauferrol A (**1**).

C. ferrea Mart (Leguminosae; Brazilian ironwood; local name Pau ferro) is a medium-sized tree found in Brazil that has traditionally been used for many medicinal purposes, such as bronchitis, diabetes, and the treatment of wounds.[9] Samples of *C. ferrea* Mart were collected in Amazonas State, Brazil in 2000. An acetone extract of the stems showed potent inhibitory activity against topo II and cell proliferation in HL60 cells. Repeated chromatography yielded pauferrol A (**1**) as an active compound.

Keywords: *Caesalpinia ferrea*; Leguminosae; Chalcone; Topoisomerase; Pauferrol A; Inhibitor; Apoptosis.

* Corresponding authors. Tel.: +81 86 256 9460; fax: +81 86 256 9559; e-mail: nozaki@dbc.ous.ac.jp

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Stems (5 kg) of *C. ferrea* Mart. were kept in acetone (18 L) at room temperature for four weeks. The solvent was removed under reduced pressure, and the residue was suspended in water, then partitioned successively with *n*-hexane, EtOAc, and BuOH. The EtOAc extracts showed significant topo II inhibitory activity at 100 μg/ml. The EtOAc extract (50 g) was subjected to silica gel column chromatography, eluting with a gradient of $CHCl_3$/MeOH (10:1→5:1→3:1→2:1→1:1→MeOH) to give six fractions. Active factions (2.5 g) were combined, and then purified by silica gel column chromatography with gradient elution ($CHCl_3$/MeOH/water = 9:1:0.5→6:1:0.1→10:2:0.1, by vol.) to give an active subfraction (388 mg), which was further purified by octadecylsilane (ODS) medium-pressure liquid chromatography (MeOH/water 5:1, v/v) and silica gel flash column chromatography (*n*-hexane/acetone 2:3, v/v) to give pauferrol A (**1**: 7.7 mg).

Pauferrol A (**1**),[10] a yellow amorphous powder (mp 174–175 °C; $[\alpha]_D$ +211; *c* 0.1, MeOH), showed a pseudo-molecular ion $[M+H]^+$ at *m/z* 767.2143 in HR-FABMS, corresponding to a molecular formula of $C_{45}H_{34}O_{12}$. The ^1H NMR and ^1H–^1H COSY spectra showed the presence of three phenolic protons (δ_H 12.98, 12.66, and 12.86) H-bonded with a ketone, three sets of aromatic protons (rings A_1, B_1, and C_1) derived from 4-hydroxyphenyl groups, two sets of aromatic protons (H-12′, H-14′, and H-15′ in ring B_2 and H-12″, H-14″, H-15″ in ring C_2) coupled in an ABX system due to 2,4-dihydroxyphenyl groups, two *ortho*-coupled aromatic protons (H-11 and H-12 in ring A_2) on a 1,2,3,4-tetrasubstituted benzene ring, a mutually coupled aliphatic proton (H-7′ and H-8′) and four aliphatic methine protons (H-7, H-8, H-7″, and H-8″). These results, together with a degree of unsaturation of 29 and three carbonyl carbon signals (δ_C 202.2, 203.6, and 203.0) in ^{13}C NMR, suggested that **1** was a chalcone trimer containing one ring system and one cyclic ether system in the molecule. The analysis of 2D NMR spectra (Fig. 1), including ^1H–^1H, HMQC, and HMBC spectra, allowed the assignment of all proton and carbon signals as shown in Table 1. The HMBC correlations [H-7′/C-2′(C-6′), C-9′, H-8′/C-1′, C-7′, C-9′, C-11, C-12, C-13: H-15′/C-9′: H-15/C-9] indicated that the chalcone unit B fused with the aromatic ring A_2 in chalcone

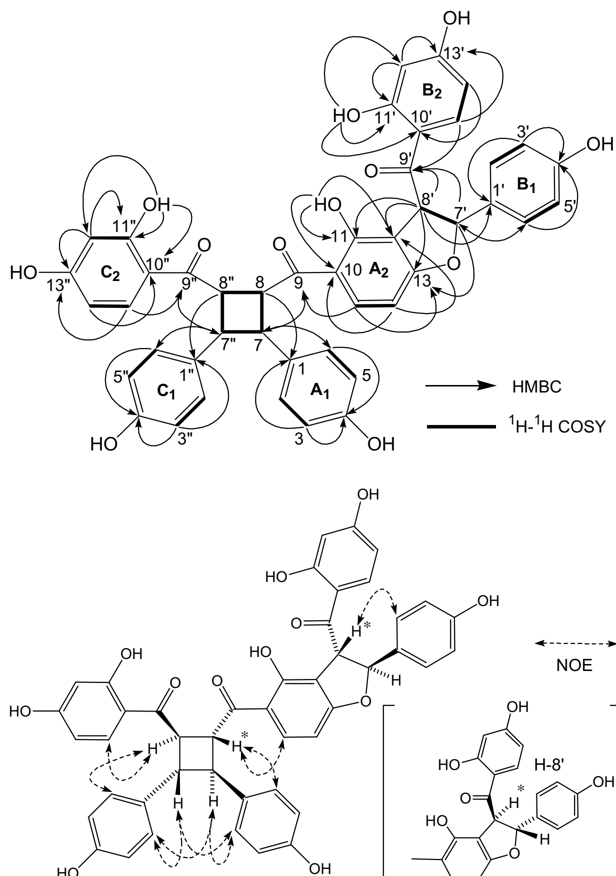

Figure 1. HMBC, ^1H–^1H COSY and NOE correlations of **1**. * The configuration between H-8 and H-8′ was not assigned.

Table 1. ^1H and ^{13}C NMR data of pauferrol A (**1**)

No.	^1H NMR	^{13}C NMR
1		132.8[a]
2, 6	7.22, 2H, d, J = 8.5 Hz[f]	129.5
3, 5	6.79, 2H, d, J = 8.5 Hz[g]	116.3[b]
4		157.5[c]
7	3.82, 1H, ddd, J = 1.2, 7.8, 8.8 Hz	49.1
8	4.57, 1H, ddd, J = 1.2, 7.8, 8.8 Hz	47.8[d]
9		203.6
10		114.5
11		161.8
11-OH	12.98, 1H, s (OH)	
12		114.1
13		168.4
14	6.29, 1H, d, J = 8.8 Hz	102.6
15	7.57, 1H, d, J = 8.8 Hz	135.6
1′		131.4
2′, 6′	7.24, 2H, d, J = 8.5 Hz	128.7
3′, 5′	6.84, 2H, d, J = 8.5 Hz	116.4[b]
4′		159.0
7′	5.90, 1H, d, J = 6.3 Hz	91.5
8′	5.38, 1H, d, J = 6.3 Hz	54.8
9′		202.2
10′		113.7
11′		166.9
11′-OH	12.66, 1H, s (OH)	
12′	6.36, 1H, d, J = 2.4 Hz	103.5[e]
13′		167.1
14′	6.38, 1H, dd, J = 8.9, 2.4 Hz	109.1
15′	7.72, 1H, d, J = 8.9 Hz	134.4
1″*		132.9[a]
2″, 6″	7.25, 2H, d, J = 8.5 Hz[f]	129.5
3″, 5″	6.82, 2H, d, J = 8.5 Hz[g]	116.4[b]
4″		157.6[c]
7″	3.84, 1H, ddd, J = 1.2, 7.8, 8.8 Hz	49.1
8″	4.53, 1H, ddd, J = 1.2, 7.8, 8.8 Hz	48.0[d]
9″		203.0
10″		113.1
11″		166.6
11″-OH	12.86, 1H, s (OH)	
12″	6.31, 1H, d, J = 2.4 Hz	103.6[e]
13″		166.1
14″	6.19, 1H, dd, J = 8.8, 2.4 Hz	108.7
15″	7.44, 1H, d, J = 8.8 Hz	134.1

NMR spectra were recorded by 500 MHz NMR in acetone-d_6 and TMS was used as internal standard.
[a–g] The signals are interchangeable.

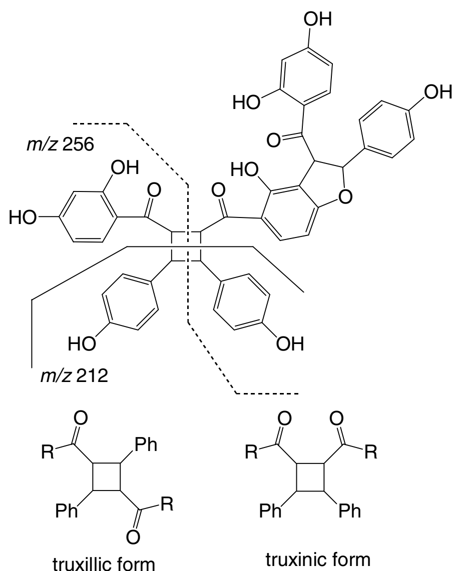

Figure 2. Mass fragment ions of **1**.

unit A to form a dihydrobenzofuran ring. Further HMBC correlations revealed the connections around the remaining four methine protons [H-7/C-2 (C-6), C-9: H-8/C-1: H-7″/ C-2″(C-6″), C-9″: H-8″/C-1″: H-15″/ C-9″]. These correlations suggested that C-7, C-8, and C-7″, C-8″ carbon atoms in chalcone units A and C were connected to form a cyclobutane moiety, supported also by ^{1}H–^{1}H COSY correlations. In the EI-MS spectrum, the characteristic fragment ions derived from cleavage of the cyclobutane moiety were observed at *m*/*z* 212 and 256 as shown in Figure 2.[11,12] Collectively, compound **1** should have the truxinic structure, consistent with the above-mentioned HMBC correlations. Therefore, a planar structure of **1** was determined, as shown in Figure 1.

The relative stereostructure was elucidated by a NOESY experiment (Fig. 1). NOE interaction was observed between H-2′ (6′) and H-8′, indicating that the configuration between H-7′ and H-8′ was trans. NOEs appeared also for H-2″/H-8″ and H-2/H-8, suggesting that H-7″ and H-8″ were trans-oriented, and H-7 and H-8 were also trans-oriented. NOEs observed for H-2″/H-7 and H-2/ H-7″ indicated that H-7 and H-7″ were trans-oriented. In addition to these results, the coupling values ($J = 7.8$ Hz and 8.8 Hz) of methine protons in cyclobutane showed that the configuration of cyclobutane protons was all-trans.[13] The relative configuration between H-8 and H-8′ could not be determined by NOEY analysis due to no correlation signal between cyclobutane and dihydrofuran rings (Fig. 1), and the limited amount of **1** prevented the chemical modification of **1** for X-ray crystal analysis. To our knowledge, this is the first example of a calchone trimer isolated from a natural source.

The inhibitory activity of pauferrol A against topo II was evaluated by determining the inhibitory effect against the decatenation activity of topo II on kinetoplast DNA.[14] Compound **1** was used at various concentrations in the topo II assay. The IC_{50} value was determined from at least three individual experiments, with three replicates for each concentration. Doxorubicin is used as a clinical anti-cancer agent and exhibits potent topo II inhibition. Under our topo II assay conditions, doxorubicin had an IC_{50} value of 0.7 μM. Compound **1** showed significant inhibitory activity (IC_{50} value 2.1 μM) under the same conditions. We examined the effect of **1** on cell proliferation in human leukemia HL60 cells. As expected, **1** exhibited a significant inhibitory effect on HL60 cell proliferation, with an IC_{50} value of 5.2 μM. The HL60 cells treated with **1** displayed characteristics of apoptosis, such as nuclear condensation and fragmentation, and DNA ladder formation, suggesting **1** induced apoptosis of HL60 cells and consequently inhibited cell proliferation. No chalcone derivatives has been reported to be a topo enzyme inhibitor before, and it is the first chalcone, an unusual trimer, with topo II inhibitory and apotosis-inducing activity. Thus, we anticipate that this chalcone will be a new lead compound for an anti-cancer agent. Compound **1** will be further investigated to determine the association between topo II inhibition and induction of apoptosis by **1**.

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10. Pauferrol A (**1**), light yellow powder; mp 174–175 °C; $[\alpha]_D^{25}$ +211 (*c* 0.1, MeOH); UV (MeOH) λ max nm (log ε): 219.8 (4.63), 226.4 (4.57), 285.0 (4.49), 317.2 (4.27); IR (KBr) ν max: 3394, 2925, 1701, 1631, 1517, 1487, 1442, 1365, 1234, 1174, 971 cm^{-1}; EI-MS *m*/*z* (rel. int.%): 319 (13), 284 (23), 256 (31), 244 (19), 212 (86), 165 (27), 148 (44), 128 (100), 107 (75); HR-FABMS *m*/*z*: 767.2143 $[M+H]^+$ (calcd for $C_{45}H_{35}O_{12}$, Δ 1.5 mmu).
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