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ANTI-PLATELET AGGREGATION CONSTITUENTS FROM FORMOSAN *TODDALIA ASIATICA*

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Key Word Index—*Toddalia asiatica*; Rutaceae; wood; toddanin; coumarin; (−)-isocoreximine; tetrahydroprotoberberine; cyclohexylamine; benzo[*c*]phenanthridine; alkaloids; lignan; anti-platelet aggregation.

Abstract—Examination on the wood of Formosan *Toddalia asiatica* led to the isolation of 30 compounds, including coumarins, alkaloids, a benzoquinone and an amine. Among the isolates, (±)-toddanin and (−)-isocoreximine are new compounds, while cyclohexylamine was isolated for the first time from nature. The structures of the compounds were elucidated from spectroscopic data and chemical evidence. Bioassay-guided fractionation led to the isolation of seven compounds showing strong anti-platelet aggregation activity *in vitro*.

INTRODUCTION

In previous reports, toddaquinoline [1], toddasiatin [2] and new *N*-cyclohexylamides [2], as well as known compounds [3], were isolated from the root bark and root wood of *Toddalia asiatica*. The authors reinvestigated this plant as the methanol extract of the wood of this species showed strong anti-platelet aggregation activity *in vitro*. Examination of the wood led to the isolation of 30 compounds, including a new pyranocoumarin, toddanin (**1**), a new tetrahydroprotoberberine alkaloid, (−)-isocoreximine (**2**), and cyclohexylamine (**3**). Compound **3** was isolated for the first time from nature. This paper describes the structural elucidation of the new compounds and the anti-platelet aggregation constituents.

RESULTS AND DISCUSSION

The $[M]^+$ of compound **1**, *m/z* 276 corresponding to $C_{15}H_{16}O_5$ was determined by EI and HR mass spectrometry. The UV spectrum showed maximal absorptions at 230, 258 sh, 304 sh and 348 nm, similar to that of braylin (**4**) (227, 258 sh, 306 sh and 354 nm), indicated that it was a 6,7-deoxygenated coumarin [4]. The IR spectrum indicated the presence of hydroxyl (3500 cm^{-1}) and a lactonic carbonyl group (1710 cm^{-1}). The ^1H NMR spectrum of **1** was also similar to that of braylin (**4**) [5], suggesting an angular 6-methoxypyranocoumarin skeleton. However, the methylene protons [δ 2.98 (1H, *dd*, J = 17.7, 5.1 Hz), 3.17 (1H, *dd*, J = 17.7, 5.1 Hz)] and an oxymethine proton [δ 3.91 (1H, *dt*, J = 6.6, 5.1 Hz collapsed to *t*, J = 5.1 Hz on addition of D_2O)], with a hydroxyl group [δ 1.82 (1H, *d*, J = 6.6 Hz, exchangeable with D_2O)] in **1**, replaced the olefinic protons H-4′ and H-3′ in braylin (**4**). Due to no identity with the linear dihydropyranocoumarin, arnottianin (**5**) [6], and by consideration of biogenesis, such as lomatin (**1a**) [7], the methylene protons were assigned to H-4′ and the oxymethine proton with the hydroxyl group to C-3′. According to the above evidence, the structure of **1** was elucidated as 3′-hydroxy dihydrobraylin, namely toddanin, and further confirmed using NOE difference experiments (Fig. 1). ORD measurements of **1** showed a plane curve, revealing toddanin to be a racemic mixture.

Compound **2** was isolated as light yellowish prisms and its molecular formula, $C_{19}H_{21}NO_4$ was determined by EI ($[M]^+$, *m/z* 327) and HR mass spectrometry. The UV spectrum showed maximal absorptions at 225 and 286 nm, and a bathochromic shift in alkaline solution, indicating the presence of a phenolic tetrahydroprotoberberine skeleton. The IR spectrum indicated the presence of hydroxyl groups (3500 and 3650 cm^{-1}) The ^1H NMR spectrum of **2** showed two methoxyl signals at δ 3.88 and 3.91 (each 3H, *s*), two phenolic hydroxyl groups at δ 5.52 (2H, *br s*), four aromatic protons at δ 6.68, 6.56 (each 1H, *s*) and δ 6.72

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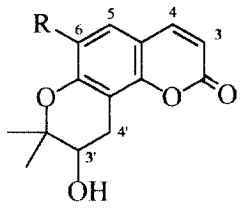

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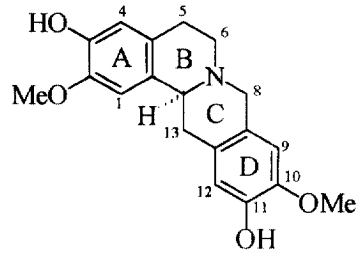
1a R = H



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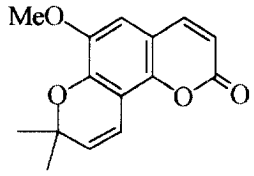

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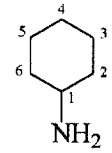

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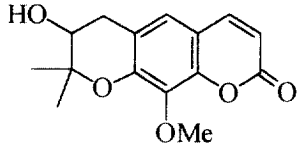

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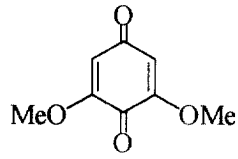

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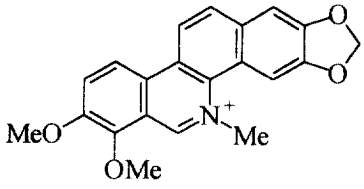

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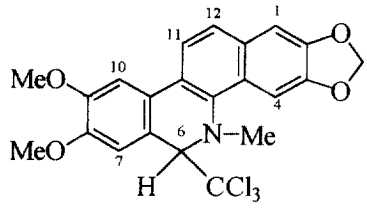

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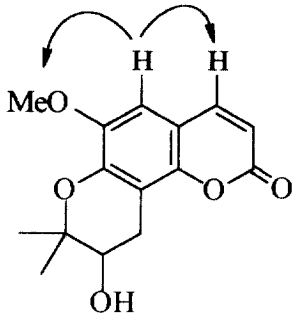

Fig. 1.

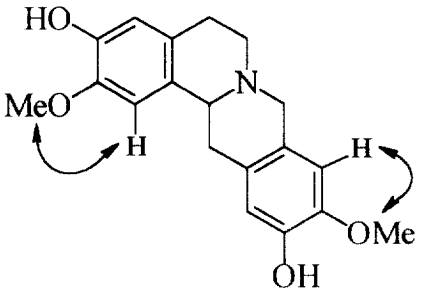

Fig. 2.

(2H, *s*), indicating a 2,3,10,11-tetraoxygenated tetrahydroprotoberberine skeleton [8]. Mass fragments at *m/z* 178, 176, 150 and 135 could indicate the pattern of oxygenated substituents of the A and D rings [9], each of which have a hydroxyl and a methoxyl groups. However, there was no $[M\text{-}OCH_3]^+$ fragment, indicating that the methoxyl group was not at the C-9 position [10]. Thus, there are four possible isomers coinciding with the fragment pattern. By comparison of the ^1H NMR data of **2** with govadine [8], coreximine [8], 3,10-dihydroxy-2,11-dimethoxy-tetrahydroprotoberberine [8] and synthetic *dl*-3,11-dihydroxy-2,10-dimethoxy-tetrahydroprotoberberine (*dl*-isocoreximine) [10], **2** was identified as isocoreximine. The structure of **2** were further confirmed by a NOESY experiment (Fig. 2), which was measured in CD_3OD to observe the H-1 [δ 6.84 (1H, *s*)] correlated with 2-OMe [δ 3.78 (3H, *s*)] and H-9 [δ 6.68 (1H, *s*)] correlated with 10-OMe [δ 3.73 (3H, *s*)]. Though *dl*-3,11-dihydroxy-2,10-dimethoxy-tetrahydroprotoberberine was previously synthesized by Brochmann-Hanssen *et al.* [11] and, recently, they revised coramine [12] which was once elucidated as **2**, into 2,11-dihydroxy-3,10-dimethoxy-tetrahydroprotoberberine (coreximine) [10]. The laevorotatory isocoreximine (**2**), with the *S*-configuration at C-14 [13] was obtained for the first time from nature.

Compound **3** showed a $[M]^+$ at *m/z* 99 which was determined by EI mass spectrometry. Its ^1H NMR spectrum was similar to that of the cyclohexyl moiety of *N*, *N'*-dicyclohexyloxamide [2] and showed 10 cyclic methylene protons at δ 1.12–1.65 (6H, *m*), 1.80 and 2.14 (each 2H, *m*). There was a cyclic methine proton at δ 3.15 (1H, *m*) and two amino protons at δ 8.23 (2H, *br s*). By comparison of the mp and IR with the synthetic HCl salt of commercial cyclohexylamine proved that **3** was cyclohexylamine, isolated as the HCl salt during the extraction process. Four *N*-cyclohexylamido derivatives were previously obtained from the root wood of this species [2]. The existence of cyclohexylamine (**3**) gave a good explanation for the biogenesis of these compounds, possibly via condensation with a cinnamic acid.

The presence of three benzo[*c*]phenanthridinium alkaloids of chelerythrine (**6**), avicine (**7**) and nitidine (**8**) was established by reduction of the mixture of quaternary bases to their respective dihydrobases [1, 2], from which the chlorides of **6**–**8** were prepared, respectively, via oxidation by DDQ. In addition, braylin (**4**) [5], nitidine $CHCl_3$ adduct (**9**), norchelerythrine (**10**) [1], isoprimpinellin (**11**) [1], phellopterin (**12**) [3], dictamnine (**13**) [14], γ-fagarine (**14**) [15], toddanone (**15**) [1], 5,7,8-trimethoxycoumarin (**16**) [1], oxynitidine (**17**) [2], (+)-toddanol (**18**) [1], (+)-6-(2-hydroxy-3-methoxy-3-methylbutyl)-5,7-dimethoxycoumarin (**19**) [1], oxychelerythrine (**20**) [2], 4-methoxy-1-methyl-2-quinolone (**21**) [1], arnottianamide (**22**) [3], integriamide (**23**) [16], (+)-toddalolactone (**24**) [1], (+)-peucedanol methyl ether (**25**) [17], 2,6-dimethoxy-*p*-benzoquinone (**26**) [16], *dl*-syringaresinol (**27**) [16], haplopine (**28**) [15], oxyterihannine (**29**) [18], *dl*-lyoniresinol (**30**) [19] were also isolated. Compounds **23**, **25**, **26**, **28**, **29** and **30** are new constituents from *Toddalia*; **9** is considered to be an artefact formed during solvent extraction. The known compounds were identified by comparison of their IR, TLC and mp with authentic samples or reference data.

The methanol extract of the wood showed strong anti-platelet aggregation activity *in vitro* using the turbidimetric method [20]. Bioassay-guided fractionation led to the isolation of chelerythrine (**6**) Cl [21], dictamnine (**13**) [14], 4-methoxy-1-methyl-2-quinolone (**21**) [14], haplopine (**28**) [15], γ-fagarine (**14**) [15], 2,6-dimethoxy-*p*-benzoquinone (**26**) and braylin (**4**), showing complete inhibitory activity at 100 μg ml^{-1} on platelet aggregation induced by 100 μm arachidonic acid *in vitro* (Table 1). Chelerythrine (**6**) Cl is an inhibitor of thromboxane formation and phosphoinositide-breakdown on rabbit platelet aggregation and the ATP release reaction [21]. 2,6-Dimethoxy-*p*-benzoquinone (**26**) also showed strong inhibition of platelet aggregation induced by collagen and PAF, and even at 10 μg ml^{-1} still exhibited complete inhibition of platelet aggregation induced by collagen. Of the coumarin isolates, only braylin (**4**) showed strong anti-platelet aggregation activity. This observation, and the results from the previous studies [22, 23], revealed that the 7-substituent (not OH) or 7,8-disubstituents of natural coumarins appear to be important for anti-platelet aggregation activity *in vitro*.

EXPERIMENTAL

Mps: uncorr. Chemical shifts in NMR: δ, with TMS as int. standard. MS: direct inlet system. UV spectra: EtOH. IR: KBr.

Plant material

Toddalia asiatica (L.) Lam. (*T. aculeata* Pers.) was re-collected at Manchou, Pingtung Hsien, Taiwan in September 1990. A voucher specimen is deposited in the Herbarium of the School of Pharmacy, Kaohsiung Medical College, Taiwan, Republic of China.

Table 1. Effect of compounds isolated from *Toddalia asiatica* on the platelet aggregation induced by ADP, thrombin, arachidonic acid, collagen and PAF. Values are presented as mean ± S.E.(*n*)

		% aggregation				
	Concn (μg ml^{-1})	ADP (20 μM)	Thr. (0.1 U ml^{-1})	AA (100 μM)	Col. (100 μg/ml^{-1})	PAF (2 ng/ml^{-1})
Control		87.6 ± 4.2(3)	90.8 ± 0.6(3)	88.5 ± 5.1(3)	89.9 ± 1.8(3)	91.2 ± 1.3(3)
(−)-Isocoreximine (**2**)	100	—	85.5 ± 2.1(5)	79.9 ± 0.4(3)	81.7 ± 1.1(3)	80.6 ± 2.1(3)
Cyclohexylamine (**3**) HCl	100	—	90.3 ± 1.0(3)	88.5 ± 0.1(3)	87.9 ± 1.4(3)	86.7 ± 0.9(3)
Braylin (**4**)	100	—	—	0.0 ± 0.0(3)***	14.8 ± 3.1(3)***	69.9 ± 7.5(3)**
Chelerythrine (**6**) Cl	100	0.0 ± 0.0(3)***	—	0.0 ± 0.0(3)***	0.0 ± 0.0(4)***	0.0 ± 0.0(3)***
	25	0.0 ± 0.0(3)***	—	0.0 ± 0.0(3)***	0.0 ± 0.0(4)***	0.0 ± 0.0(3)***
Isopimpinellin (**11**)	50	74.8 ± 4.3(3)		20.4 ± 17.6(3)**	93.4 ± 2.7(3)	86.5 ± 5.1(3)
Phellopterin (**12**)	100	Caused platelet aggregation without any inducer				
Toddanone (**15**)	100	70.5 ± 11.7(3)	—	44.8 ± 13.5(3)*	81.8 ± 3.7(3)	86.3 ± 3.0(3)
5,7,8-Trimethoxycoumarin (**16**)	100	—	89.7 ± 0.9(3)	71.8 ± 6.3(3)*	86.5 ± 1.2(3)*	89.2 ± 1.0(3)*
Toddanol (**18**)	100	—	91.3 ± 0.4(3)	81.5 ± 3.1(3)*	87.5 ± 1.3(3)	86.9 ± 1.8(3)*
6-(2-Hydroxy-3-methoxy-3-methyl-butyl)-5,7-dimethoxycoumarin (**19**)	100	—	90.1 ± 0.2(3)	90.3 ± 0.5(3)	89.0 ± 1.0(3)	81.6 ± 3.9(3)
Toddalolactone (**24**)	100	91.1 ± 0.6(3)	—	87.5 ± 0.9(3)	86.4 ± 2.1(3)	89.9 ± 0.3(3)*
Peucedanol methyl ether (**25**)	100	—	89.2 ± 0.9(3)	84.1 ± 0.4(3)	87.7 ± 0.8(3)	85.8 ± 2.5(3)
2,6-Dimethoxy-*p*-benzoquinone (**26**)	100	—	24.2 ± 1.5(5)***	0.0 ± 0.0(3)***	0.0 ± 0.0(3)**	0.0 ± 0.0(3)***
	50	—	25.0 ± 2.9(4)***	0.0 ± 0.0(3)***	0.0 ± 0.0(3)***	0.0 ± 0.0(3)***
	20		47.7 ± 7.1(4)***	9.3 ± 7.6(3)***	0.0 ± 0.0(3)***	11.7 ± 5.01(3)***
	10	—	71.9 ± 9.6(4)*	56.6 ± 7.5(3)***	0.0 ± 0.0(3)***	45.4 ± 18.6(3)***
dl-Syringaresinol (**27**)	100	64.9 ± 12.9(3)		44.8 ± 13.5(3)	83.9 ± 4.9(4)	82.7 ± 1.0(3)*

Platelets were preincubated with compounds isolated from *T. asiatica* or DMSO (0.5%, control) at 37° for 3 min, then inducer ADP (or thrombin), arachidonic acid (AA), collagen and PAF added.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ as compared with respective control.

Extraction and separation

Dried wood (24.6 kg) was extracted with warm MeOH and the extract evapd to dryness. A large ppt. (36.5 g) was removed by filtration when the MeOH soln was concd *in vacuo*. The filtrate was concd then triturated with 5% HOAc to produce acid-sol. and -insol. frs (600 g). The acidic soln was processed as described in Ref. [1], to divide into base reineckate (141 g), yellow ppt. of benzol[*c*]phenanthridinium chloride (5.7 g), tert. nonphenolic bases (126 g) and tert. phenolic bases (12.1 g). Part of the tert. nonphenolic bases (63.3 g) was subjected to CC on silica gel, eluting with $CHCl_3$ gradually enriched with MeOH, to obtain 6 crystalline frs (1–6). Fr. 1 ($CHCl_3$, 0.24 g) afforded **9** (3.8 mg) and the filtrate was resubjected to CC, eluting with *n*-hexane to obtain **10** (87 mg). Fr. 2 ($CHCl_3$ 2.96 g) after washing with Et_2O gave **11** (1.58 g) and the washings resubjected to CC, eluting with *n*-hexane gradually enriched with EtOAc to provide 2 frs (2-1–2-2). Fr. 2-1 [*n*-hexane–EtOAc (20 : 1), 0.45 g] washed with MeOH yielded **12** (72 mg). The washings (0.38 g) were resubjected to CC, eluting with benzene and EtOAc to afford **13** benzene–EtOAc (20 : 1), 50 mg] and **4** [benzene-EtOAc (1- : 1), 0.21 g]. Fr. 2-2 [*n*-hexane-EtOAc (5 : 1), 76.1 mg] furnished **14** (36.1 mg) by prep. TLC [benzene-EtOAc (5 : 1)]. Fr. 3 [$CHCl_3$–MeOH (100 : 1), 1.21 g] washed with Et_2O gave **15** (61.3 mg). The mother liquor (0.296 g) was subjected to CC, eluting with benzene gradually enriched with EtOAc. Recrystallization of the crystalline eluate [benzene–EtOAc (20 : 1)] from $CHCl_3$–MeOH afforded **16** (19.2 mg). The Et_2O washings (1.10 g) from fr. 3 were subjected to CC, eluting with benzene gradually enriched with EtOAc to provide 2 frs (2-3-1–2-3-2). Fr. 2-3-1 [benzene–EtOAc (10 : 1), 0.623 g] furnished **17** (4 mg). Fr. 2-3-2 [benzene EtOAc (10 : 1), 0.329 g] yielded **18** (231 mg). Fr. 4 [$CHCl_3$–MeOH (20 : 1), 29.42 g] washed with MeOH gave **19** (6.79 g). The MeOH washings (22.6 g) were resubjected to CC, eluting with benzene gradually enriched with EtOAc to provide 2 crystalline frs (4-1–4-2). Fr. 4-1 [benzene–EtOAc (20 : 1), 0.734 g] washed with MeOH and recrystallized gave **20** (3.5 mg). The washings (0.692 g) were resubjected to CC, eluting with *n*-hexane and EtOAc to afford **21** [*n*-hexane–EtOAc (5 : 1), 33.1 mg]. Fr. 4-2 [benzene–EtOAc (10 : 1), 2.353 g] washed with Et_2O and subjected to CC, eluting with $CHCl_3$ and MeOH, yielded **22** [$CHCl_3$–MeOH (20 : 1), 3.1 mg]. The mother liquor was resubjected to CC and purified by prep. TLC [$CHCl_3$–MeOH (20 : 1)] to furnish **23** (3.5 mg). The Et_2O washings (76.5 mg) from fr. 4-2 were resubjected to CC and purified by prep. TLC [$CHCl_3$–MeOH (20 : 1)] to furnish **1** (3.5 mg). Fr. 5 [$CHCl_3$–MeOH (10 : 1), 23.7 g] washed with MeOH and recrystallized from MeOH gave **24** (11.2 g). The MeOH washings were resubjected to CC and purified by prep. TLC [$CHCl_3$–MeOH (15 : 1)] to afford **25** (5.7 mg). Fr. 6 [$CHCl_3$–MeOH (5 : 1), 0.956 g] was subjected to CC to obtain **3** [$CHCl_3$–MeOH (10 : 1), 0.154 g]. The tert. phenolic bases (12.1 g) were subjected to CC on silica gel, eluting with $CHCl_3$ gradually enriched with MeOH to provide 3 frs (1–3). Fr. 1 [$CHCl_3$–MeOH (50 : 1), 73.1 mg] washed with Et_2O and recrystallized from MeOH gave **26** (6.4 mg). Fr. 2 [$CHCl_3$–MeOH (20 : 1), 4.19 g] washed with EtOAc and subjected to CC yielded **27** (1.04 g). The EtOAc washings were resubjected to CC, eluting with $CHCl_3$ gradually enriched with EtOAc to provide 3 frs (2-1–2-3). Fr. 2-1 [$CHCl_3$–EtOAc (20 : 1), 0.229 g] washed with EtOAc and resubjected to CC furnished **24** (67.6 mg) and **23** (6.5 mg). Fr. 2-2 [$CHCl_3$–EtOAC (5 : 1), 0.164 g] washed with Et_2O and subjected to CC afforded **28** (22.4 mg). The washings were resubjected to CC, eluting with benzene and EtOAc to obtain **29** [C_6H_6–EtOAc (5 : 1), 2.5 mg]. Fr. 2-3 [$CHCl_3$–EtOAc (1 : 1), 0.164 g] was resubjected to CC, eluting with CH_2Cl_2 and EtOAc to furnish **2** [CH_2Cl_2–EtOAc (3 : 1), 7.1 mg]. Fr. 3 (2.842 g) was subjected to CC, eluting with CH_2Cl_2 gradually enriched with EtOAc to provide 2 frs (3-1–3-2). Fr. 3-2 [CH_2Cl_2–EtOAc (2 : 1), 0.535 g] washed with EtOAc and recrystallized yielded **30** (46.3 mg). The quat. benzo[*c*]phenanthridinium mixt. (5.7 g) was reduced with $NaBH_4$–MeOH to the tert. bases and then subjected to CC on silica gel, eluting with *n*-hexane gradually enriched with EtOAc to provide 3 frs (1–3). Fr. 1 (*n*-hexane, 1.803 g) was washed with MeOH and recrystallized to give dihydroavicine (1.303 g). Fr. 2 [*n*-hexane–EtOAc (100 : 1), 0.963 g] washed with MeOH and recrystallized afforded dihydrochelerythrine (0.655 g). Fr. 3 [*n*-hexane–EtOAc (50 : 1), 1.544 g] washed with MeOH and recrystallized gave dihydronitidine (1.265 g).

*Toddanin (**1**)*

Light yellowish prisms (MeOH), mp 219–221°. UV λ_{max} nm (log ε): 230 (3.72), 258 sh (3.04), 304 sh (3.27), 348 (3.58). IR ν_{max} cm^{-1}: 3500 (OH), 1710 (CO). EIMS *m/z* (rel. int.): 276 $[M]^+$ (65), 243 (22), 206 (100). HRMS: $C_{15}H_{16}O_5$. Found: 276.0991, calc. 276.0998. ^1H NMR (200 MHz, $CDCl_3$): δ 1.41 (3 H, *s*, Me), 1.48 (3H, *s*, Me), 1.82 (1H, *d*, J = 6.6 Hz, OH, exchangeable with D_2O), 2.98 (1H, *dd*, J = 17.7, 5.1 Hz, H-4′β), 3.17 (1H, J = 17.7, 5.1 Hz, H-4′α), 3.90 (3H, *s*, OMe), 3.91 (1H, *dt*, J = 6.6, 5.1 Hz, collapsed to *t*, J = 5.1 Hz on addition of D_2O, H-3′), 6.28 (1H, *d*, J = 9.4 Hz, H-3), 6.77 (1H, *s*, H-5), 7.61 (1H, *d*, J = 9.4 Hz, H-4).

*(−)-Isocoreximine (**2**)*

Light yellowish prisms ($CHCl_3$–MeOH), mp 238–242°. $[\alpha]_D^{25}$: −361.0° (MeOH, ca 0.1) UV λ_{max} nm (log ε): 225 (3.18), 286 (2.93). IR ν_{max} cm^{-1}: 3500, 3650 (OH). EIMS *m/z* (rel. int.): 327 $[M]^+$ (51), 178 (100), 176 (51), 150 (69), 135 (28). HRMS: $C_{19}H_{21}NO_4$. Found: 327.1468, calc. 327.1471. ^1H NMR (200 MHz, $CDCl_3$): δ 3.88 (3H, *s*, 10-OMe), 3.91 (3H, *s*, 2-OMe),

5.52 (2H, *br s*, OH, disappeared after addition of D_2O), 6.56 (1H, *s*, H-12), 6.68 (1H, *s*, H-4), 6.72 (2H, *s*, H-1,9). ^{1}H NMR (200 MHz, CD_3OD): δ 3.73 (3H, *s*, 10-OMe), 3.78 (3H, *s*, 2-OMe), 6.52 (1H, *s*, H-12), 6.58 (1H, *s*, H-4), 6.68 (1H, *s*, H-9), 6.84 (1H, *s*, H-1).

Cyclohexylamine (3) HCl

Colourless prisms (Et_2O–MeOH), mp 210–212°. EIMS *m/z* (rel. int.): 99 $[M]^+$ (42), 70 (36), 56 (100). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.12–1.65 (6H, *m*, $CH_2 \times 3$), 1.80 (2H, *m*, H-2β,6β), 2.14 (2H, *m*, H-2α,6α), 3.15 (1H, *m*, H-1), 8.23 (2H, *br s*, NH_2).

Nitidine $CHCl_3$ adduct (9)

Colourless prisms (MeOH), mp 202–206°. UV λ_{max} nm (log ε): 285 (4.64), 303 sh (4.40), 329 (4.38). IR ν_{max} cm^{-1}: 945, 1040 (OCH_2O). FAB-HRMS *m/z*: $C_{22}H_{18}NO_4Cl_3$: $[M+H]^+$, Found: 466.0380; calc. 466.0382. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.01 (3H, *s*, NMe), 3.98 (3H, *s*, OMe), 4.04 (3H, *s*, OMe), 4.72 (1H, *s*, H-6), 6.07, 6.08 (each 1H, *d*, $J = 1.2$ Hz, OCH_2O), 7.12 (1H, *s*, H-1), 7.17 (1H, *s*, H-10), 7.42 (1H, *s*, H-4), 7.46 (1H, *d*, $J = 8.6$ Hz, H-12), 7.71 (1H, *s*, H-7), 7.72 (1H, *d*, $J = 8.6$ Hz, H-11).

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REFERENCES

1. Chen, I.-S., Tsai, I.-L., Wu, S.-J., Sheen, W.-S., Ishikawa, T. and Ishii, H., *Phytochemistry*, 1993, **34**, 1449.
2. Tsai, I.-L., Fang, S.-C., Ishikawa, T., Chang, C.-T. and Chen, I. S., *Phytochemistry*, 1997, **44**, 1383.
3. Tsai, I-L., Chang, R.-G., Fang, S.-C., Ishikawa, T. and Chen, I. S., *Chinese Pharmaceutical Journal*, 1996, **48**, 63.
4. Murray, R. D. H., Mendez, J. and Brown, S. A., *The Natural Coumarins*. Wiley and Sons, New York, 1982, p. 28.
5. Reisch, J. and Strobel, H. *Pharmazie*, 1982, **37**, 862.
6. Ishii, H. and Hosoya, K., *Chemical and Pharmaceutical Bulletin*, 1972, **20**, 860.
7. Soine, T. O. and Jawad, F. H., *Journal of Pharmaceutical Sciences*, 1964, **53**, 990.
8. Ohiri, F. C., Verpoorte, R. and Baeheim Svendsen, A., *Planta Medica*, 1983, **49**, 162.
9. Chen, C.-Y. and MacLean, D. B., *Canadian Journal of Chemistry*, 1968, **46**, 2501.
10. Brochmann-Hanssen, E. and Chiang, H.-C., *Journal of Organic Chemistry*, 1977, **42**, 3588.
11. Chiang, H.-C. and Brochmann-Hanssen, E., *Journal of the Taiwan Pharmaceutical Association*, 1975, **27**, 90.
12. Yunosov, M. S., Akramov, S. T. and Yu, S., *Doklady Akademii Nauk SSR*, 1965, **162**, 603. [*Chemical Abstracts*, 1966, **63**, 5695].
13. Shamma, M., *The Isoquinoline Alkaloids: Chemistry and Pharmacology*. Academic Press, New York, 1972, p. 292.
14. Sheen, W.-S., Tsai, I.-L., Teng, C.-M. and Chen, I.-S., *Phytochemistry*, 1994, **36**, 213.
15. Chen, I.-S., Wu, S.-J., Lin, Y.-C., Tsai, I.-L., Seki, H., Ko, F.-N. and Teng, C.-M., *Phytochemistry*, 1994, **36**, 237.
16. Ishii, H., Chen, I.-S., Akaike, M., Ishikawa, T. and Lu, S.-T., *Yakukagku Zasshi*, 1982, **102**, 182.
17. Ishii, H. and Hosoya, K., Ishikawa, T., Ueda, E. and Haginiwa, J., *Yakugaku Zasshi*, 1974, **94**, 332.
18. Ishii, H., Ishikawa, T., Akaike, M., Tohjoh, T., Toyoki, M., Ishikawa, M. and Chen, I.-S., *Yakugaku Zasshi*, 1984, **104**, 1030.
19. Dallacker, F. and Van Wersh, H., *Chemische Berichte*, 1975, **108**, 561.
20. O'Brien, J. R., *Journal of Clinical Pathology*, 1962, **15**, 452.
21. Ko, F.-N., Chen, I.-S., Wu, S.-J., Lee, L.-G., Haung, T.-F. and Teng, C.-M., *Biochimica et Biophysica Acta*, 1990, **1052**, 560.
22. Chen, I.-S., Lin, Y.-C., Tsai, I.-L., Teng, C.-M., Ko, F.-N., Ishikawa, T. and Ishii, H., *Phytochemistry*, 1995, **39**, 1091.
23. Chen, I.-S., Chang, C.-T., Sheen, W.-S., Teng, C.-M., Tsai, I.-L., Duh, C.-Y. and Ko, F.-N., *Phytochemistry*, 1996, **41**, 525.