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N-CYCLOHEXYL AMIDES AND A DIMERIC COUMARIN FROM FORMOSAN *TODDALIA ASIATICA*

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Key Word Index—*Toddalia asiatica*; Rutaceae; root wood; *N*-cyclohexylamides; toddaliamide; methyltoddaliamide; dimeric coumarin; toddasiatin; benzo[*c*]phenanthridine; alkaloids.

Abstract—Examination of the root wood of Formosan *Toddalia asiatica* led to the isolation of four *N*-cyclohexylamides: *N*,*N*′-dicyclohexyloxamide, *N*,*N*′-dicyclohexylurea, toddaliamide, methyltoddaliamide; one dimeric coumarin: toddasiatin; and six alkaloids: skimmianine, norchelerythrine, oxyavicine, avicine, oxynitidine and nitidine, from the tertiary non-phenolic basic fraction. Toddaliamide, methyltoddaliamide and toddasiatin are new compounds while *N*,*N*′-dicyclohexyloxamide and *N*,*N*′-dicyclohexylurea were isolated for the first time from nature. The structures of all the compounds were elucidated by spectral data and chemical evidence. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

The roots and root barks of *Toddalia asiatica* (L.) Lam. (*T. aculeata* Pers.; Rutaceae) are used as a folk medicine in Asia to relieve pain and stasis as well as for haemostatics [1, 2]. We have reported the isolation of a new benzo[*h*]quinoline, toddaquinoline, along with six coumarins and six alkaloids from the tertiary basic fraction of the root bark [3] and 25 compounds from the neutral fraction of the root bark [4] of Formosan species. Recently, examination of the root wood of the same plant provided 10 alkaloids, including four *N*-cyclohexylamides: *N*,*N*′-dicyclohexyloxamide (**1**), *N*,*N*′-dicyclohexylurea (**2**), toddaliamide (**3**) and methyltoddaliamide (**4**), along with one dimeric coumarin: toddasiatin (**5**). This paper describes the isolation and structural elucidation of these compounds.

RESULTS AND DISCUSSION

The molecular ion ($[M]^+$) of compound **1**, corresponding to $C_{14}H_{24}N_2O_2$, was determined by EI mass spectrometry to be at *m*/*z* 252. Compound **2** was lacking a carbonyl group compared to **1** and had $[M]^+$ at *m*/*z* 224. The IR spectra of **1** and **2** indicated the presence of an NH group and an NHCO group. The ^{1}H NMR spectrum of **1** showed the signals for 10 cyclic methylene protons at δ 1.21 (6H, *m*), 1.33 (4H, *m*), 1.59 (2H, *m*), 1.73 (4H, *m*) and 1.88 (4H, *m*). There were two cyclic methine protons at δ 3.70 (2H, *m*) and two amido protons at δ 7.36 (2H, *br d*, J = 7.2 Hz). The ^{1}H NMR pattern of **2** was very similar to that of **1** and also showed 10 methylene protons at δ 1.12 (6H, *m*), 1.35 (4H, *m*), 1.58 (2H, *m*), 1.69 (4H, *m*) and 1.94 (4H, *m*), along with two cyclic methine protons at δ 3.48 (2H, *m*) and two amido protons at δ 4.05 (2H, *br d*, J = 7.2 Hz). From the above data, compound **1** was elucidated as *N*,*N*′-dicyclohexyloxamide and it had TLC and IR properties identical with the authentic sample, which was previously synthesized by Uemura *et al.* [5]. Compound **2** was elucidated as *N*,*N*′-dicyclohexylurea, which was also identified by comparison of its TLC and IR spectral data with those of the commercial product. Both **1** and **2** were obtained for the first time from nature.

Compound **3** was isolated as prisms and the molecular formula $C_{22}H_{33}NO_6$ was determined by elementary analysis. A mass fragment peak at *m*/*z* 389 $[M - H_2O]^+$ suggested the presence of an alcoholic hydroxyl group in the molecule. The IR spectrum indicated the presence of hydroxyl (3400 cm^{-1}), NH (3270 cm^{-1}) and NHCO (1650 cm^{-1}) groups. The UV spectrum showed maximum absorptions at 225, 245sh, 300 and 320 nm and a bathochromic shift in alkaline solution to indicate the presence of a phenolic moiety. The ^{1}H NMR spectrum of **3** showed only one aromatic proton at δ 6.50, suggestive of a benzene ring with five substituted groups. One was a *trans*-

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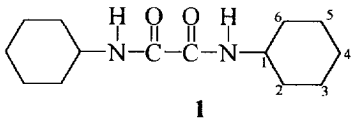

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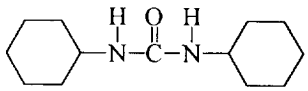

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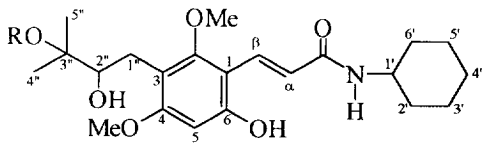
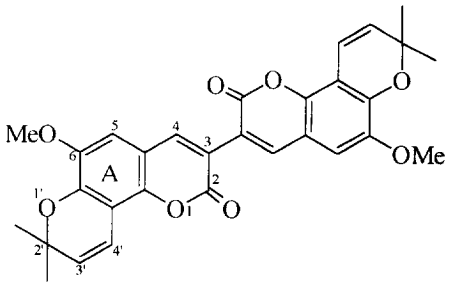


3 R=H
4 R=Me



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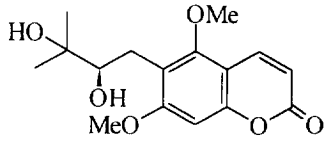

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(3-cyclohexylamino-3-oxo)-1-propenyl group, which showed a cyclohexyl-methine proton at δ 4.30 (*m*) coupled with an NH at δ 8.59 (*d*, J = 8.0 Hz) and two *trans*-olefinic protons at δ 7.80 and 8.78 (*d*, J = 15.9 Hz). The signals of a 2,3-dihydroxy-3-methylbutyl group were at δ 1.54, 1.55 (each 3H, *s*), 3.18 (1H, *dd*, J = 2.7, 13.4 Hz), 3.22 (1H, *dd*, J = 9.6, 13.4 Hz), 4.21 (1H, *br d*, J = 9.6 Hz), 5.40 (1H, *s*, OH) and 5.42 (1H, *br s*, OH). The negative result of the Gibbs test suggested that the aromatic proton was not at the *para*-position of the phenolic hydroxyl group [δ 12.08 (1H, *br s*)]. The ^{13}C NMR spectral shifts of the remaining two methoxyl groups at δ 55.3 and 61.6 allowed their assignment to C-4 and C-2, respectively. The above observation and the NOE difference (Fig. 1) suggested that **3** was the possible structure, but an alternative structure with the side chains at C-1 and

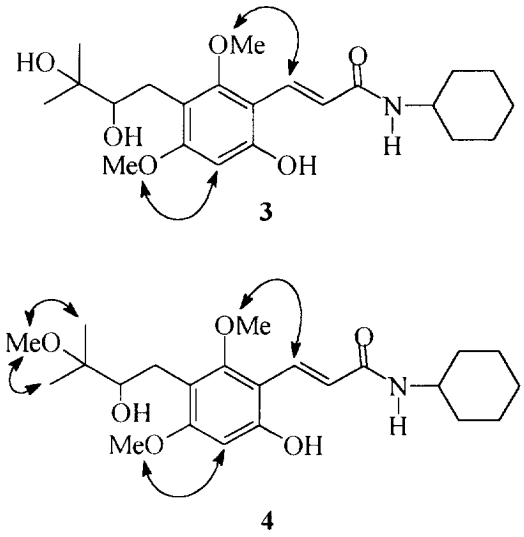

Fig. 1. NOE differences between compounds **3** and **4**.

C-3 interchanged was also possible. The ^{13}C NMR data was assigned by DEPT, HETCOR and HMBC techniques (Fig. 2), which further supported the elucidation of **3** as 3-(2,3-dihydroxy-3-methylbutyl)-6-hydroxy-2,4-dimethoxy-cinnamoyl-*N*-cyclohexylamide.

Toddalolactone (**6**) and other 5,7-dimethoxy-6-substituted coumarins are the characteristic constituents of the root bark and root wood of this plant [4, 6]. Opening the lactone ring of **6** would give an *O*-hydroxycinnamic acid derivative, which shows a similar substituted pattern on the benzene ring with that of **3**. Thus, the existence of **3** was reasonable and could arise as the amidation product of a *O*-hydroxycinnamic acid derivative with cyclohexylamine in the biogenetic process. In view of the *R*-configuration of (+)-toddalolactone (**6**) [7] and the presence of a series of its (+)-analogous [7] in the same plant, the stereochemistry at C-2″ of **3** would appear to be of the *R*-configuration, but an enantiomeric antipode was not available for comparison.

The UV and IR spectra of **4** resembled those of **3**. The molecular formula of $C_{23}H_{35}NO_6$ was determined by EI ([M]$^+$ at *m/z* 421) and HR mass spectrometry, which revealed an additional methoxyl group in **4** when compared to **3**. The ^{1}H NMR spectrum of **4** also showed an additional C-3″ methoxyl signal at δ 3.23 (3H, *s*) in place of a hydroxyl in **3**. Therefore, compound **4** was considered to be the C-3″ *O*-methyl derivative of **3**. The structure of **4** was thus elucidated as 3-(2-hydroxy-3-methoxy-3-methylbutyl)-6-hydroxy-2,4-dimethoxycinnamoyl-*N*-cyclohexylamide by

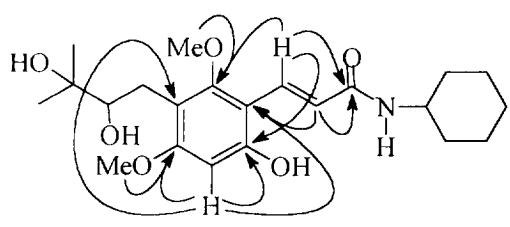

Fig. 2. HMBC of compound **3**.

the NOE experiments (Fig. 1) and from the biogenetic considerations. This is the first time **4** has been obtained in nature, and we have named it methyl toddaliamide (**4**).

Compound **5** was isolated as yellow prisms. The molecular formula of $C_{30}H_{26}O_8$ was established by HR and EI mass spectrometry, (M^+ at *m/z* 514) which indicated a dimeric coumarin with a dimethyl pyran and one methoxyl group both on ring A. The UV spectrum showed the maximum absorption at 226, 260, 270sh, 305sh and 360 nm to indicate **5** having a 6,7-dioxygented coumarin skeleton [8]. The ^{1}H NMR spectrum of **5** was very simple and suggested a symmetric biscoumarin. A dimethyl pyran ring [δ 1.54 (6H, *s*, Mex2), δ 5.77 and 6.93 (each 1H, *d*, $J = 9.9$ Hz, H-3′ and H-4′)] was recognized as an angular orientation and similar to braylin with a lower H-4′ chemical shift [9] which ruled out the possibility of a linear form such as found in lunvagetin [10]. No long-range coupling occurred at aromatic protons and the only aromatic singlet at δ 6.89 (1H) was reasonably assigned at C-5 and the methoxyl group was thus located at C-6 as in braylin [9]. The location of the dimethyl pyran ring at C-7 and C-8 was consistent with the above observations. The lack of the characteristic signal of H-3 at δ 6.2–6.30 as in a simple coumarin suggested a C3–C3 linkage between two monomer coumarin units. The reason the signal of H-4 was downfield of δ 8.53, while there was no oxygenated substituent at C-5, could be explaned by the anisotropic effect of the carbonyl group in the lactone ring of the counterpart coumarin moiety. According to the above observations, the structure of toddasiatin was elucidated as **5** and to be a dimer of braylin with a C3–C3 linkage. The available amount of **5** was too small to carry out the NOE correlation of H-4 and H-5. However, the isolation of braylin and norbraylin from this plant [9] provides good evidence for the existence of the new dimeric coumarin, toddasiatin.

The presence of two benzo[*c*]phenanthridinium alkaloids of avicine and nitidine was proved by the reduction of the mixtures of quaternary bases to dihydroavicine and dihydronitidine, respectively [3]. In addition, skimmianine [3], norchelerythrine [3], oxyavicine [4] and oxynitidine [11] were also isolated, and identified by comparison of their IR, TLC and melting point data with those of the corresponding authentic samples.

EXPERIMENTAL

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

Plant material. Toddalia asiatica was collected at Manchou, Pingtung Hsien, Taiwan, in October 1980. A voucher specimen was deposited in the Herbarium of the School of Pharmacy, Kaohsiung Medical College, Taiwan.

Extraction and separation. Dried root wood (8 kg) was extracted with warm MeOH and the extract evapd to dryness. The MeOH extract was triturated with 5% HOAc to produce acid soluble and insoluble frs. The acidic soln was made alkaline with NH_4OH, then extracted with $CHCl_3$. The $CHCl_3$ soln after extraction with aq. 5% NaOH was dried with K_2CO_3, then concd to moderate vol. The concd $CHCl_3$ soln was treated with aq. 10% HCl and the resulting yellow ppt. (benzo[*c*]phenanthridinium chloride, 1.696 g) recovered by filtration. The filtrate was made alkaline with NH_4OH and then extracted with $CHCl_3$. The $CHCl_3$ soln was dried with K_2CO_3, then concd to give a brown residue (tertiary non-phenolic base, 41.5 g). Part of the non-phenolic base (24.4 g) was subjected to CC on silica gel, eluting with $CHCl_3$ gradually enriched with MeOH, to obtain 4 frs (1–4). Crystalline fr. 1 ($CHCl_3$, 2.3 g) afforded **1** (55 mg) and norchelerythrine (2 mg). Crystalline fr. 2 ($CHCl_3$, 2.2 g) gave skimmianine (39 mg) and the mother liquor was resubjected to CC on silica gel, eluting with *n*-hexane gradually enriched with EtOAc, to provide 4 crystalline frs (2-1–2-4). Fr. 2-1 [*n*-hexane–EtOAc (7:1), 0.42 g] yielded **2** (9 mg), fr. 2-2 [*n*-hexane–EtOAc (7:1), 0.65 g] furnished oxyavicine (1 mg) and fr. 2-3 [*n*-hexane–EtOAc (1:1), 0.28 g] gave oxynitidine (3.6 mg). Fr. 3 [$CHCl_3$–MeOH (100:1), 0.21 g) was subjected to CC on silica gel, eluting with *n*-hexane gradually enriched with EtOAc, to furnish **5** (1.2 mg) after purification by prep. TLC [C_6H_6–EtOAc (2:1)] and recrystallzation from the eluate [*n*-hexane–EtOAc (4:1)]. Crystalline fr. 4 [$CHCl_3$–MeOH (5:1), 12.2 g] was subjected to CC silica gel, eluting with *n*-hexane gradually enriched with EtOAc, to divide into fr. 4-1 and fr. 4-2. From fr. 4-1 [*n*-hexane–EtOAc (2:3), 0.3 g], **4** (11.6 mg) was obtained and fr. 4-2 [EtOAc–MeOH (5:1), 5.8 g] afforded **3** (0.18 g).

Reduction of benzo[c]*phenanthridinium chloride fraction.* $NaBH_4$ (0.23 g) was added in portions to a stirred soln of a part of the benzo[*c*]phenanthridinium chloride fr. (0.2 g) in MeOH (10 ml) at room temp. After stirring at room temp. for 30 min then usual work-up a residue (0.18 g) was obtained which was subjected to CC on silica gel, eluting with *n*-hexane–EtOAc, (50:1) to yield dihydroavicine (11.2 mg); elution with *n*-hexane–EtOAc (4:1) then yielded dihydronitidine (10 mg).

N,N′-*Dicyclohexyloxamide* (**1**). Prisms ($CHCl_3$–MeOH), mp 243–248°. IR ν_{max} cm^{-1}: 3280 (NH), 1640 (NHCO). EIMS *m/z* (rel. int.): 252 $[M]^+$ (21), 224 (15), 171 (42), 128 (33), 126 (22), 98 (51), 89 (35), 83 (100), 55 (90). Anal. calc. $C_{14}H_{24}N_2O_2$: C, 39.64; H, 1.90; N, 13.21. Found: C, 39.71; H, 1.93; N, 13.23. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.21 (6 H, *m*, $CH_2 \times 3$), 1.33 (4H, *m*, $CH_2 \times 2$), 1.59 (2H, *m*, CH_2), 1.73, 1.88 (each 4H, *m*, $CH_2 \times 4$), 3.70 (2H, *m*, H-1 × 2), 7.36 (2H, *br d*, $J = 7.2$ Hz, NH × 2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.7 (C-3,5), 25.4 (C-4), 32.6 (C-2,6), 48.7 (C-1), 159.1 (CO).

N,N′-*Dicyclohexylurea* (**2**). Prisms ($CHCl_3$–MeOH), mp 230–232°. IR ν_{max} cm^{-1}. 3340 (NH), 1630

(NHCO). EIMS *m/z* (rel. int.): 224 $[M]^+$ (99), 143 (83), 99 (100), 98 (59), 83 (31), 61 (66), 55 (53), 43 (44), 41 (44). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.12 (6 H, *m*, $CH_2 \times 3$), 1.35 (4H, *m*, $CH_2 \times 2$), 1.58 (2H, *m*, CH_2), 1.69 (4H, *m*, $CH_2 \times 2$), 1.94 (4H, *m*, $CH_2 \times 2$), 3.48 (2 H, *m*, H-1 × 2), 4.05 (2H, *br d*, $J = 7.2$ Hz, NH × 2).

Toddaliamide (**3**). Prisms (MeOH–EtOAc), mp 227–228°. $[\alpha]_D^{23}$ +19.0° (MeOH, *c* 0.1). UV λ_{max} nm (log ε): 205 (4.48), 225 (4.27), 245sh (4.22), 300 (4.21), 320 (4.19). IR ν_{max} cm^{-1}. 3400 (OH), 3270 (NH), 1650 (NHCO). EIMS *m/z* (rel. int.): 389 $[M - H_2O]^+$ (2), 308 (15), 250 (33), 220 (50), 219 (100), 207 (70), 161 (24), 56 (48). Anal. calc. $C_{22}H_{33}NO_6$: C, 64.84; H, 8.16; N, 3.44. Found: C, 64.77; H, 8.10; N, 3.40. ^{1}H NMR (500 MHz, pyridine-d_5): δ 1.07 (1H, *m*, H-4′a), 1.31 (2H, *m*, H-3′a,5′a), 1.39 (2H, *m*, H-2′a,6′a), 1.49 (1H, *br d*, $J = 12.9$ Hz, H-4′b), 1.54 (3H, *s*, Me), 1.55 (3H, *s*, Me), 1.64 (2H, *br d*, $J = 12.9$ Hz, H-3′b,5′b), 2.11 (2H, *br d*, $J = 9.9$ Hz, H-2′b,6′b), 3.18 (1H, *dd*, $J = 2.7$, 13.4 Hz, H-1″a), 3.22 (1H, *dd*, $J = 9.6$, 13.4 Hz, H-1″b), 3.51 (3H, *s*, OMe-4), 3.84 (3H, *s*, OMe-2), 4.21 (1H, *br d*, $J = 9.6$ Hz, H-2″), 4.30 (1H, *m*, H-1′), 5.40 (1H, *s*, OH-3″), 5.42 (1H, *br s*, OH-2″), 6.50 (1H, *s*, H-5), 7.80 (1H, *d*, $J = 15.9$ Hz, H-α), 8.59 (1H, *d*, $J = 8.0$ Hz, NH), 8.78 (1H, *d*, $J = 15.9$ Hz, H-β), 12.08 (1H, *br s*, OH-6). ^{13}C NMR (125 MHz, pyridine-d_5): δ 25.5 (C-3′,5′), 25.6 (C-4″ or C-5″), 25.9 (C-4′), 26.3 (C-5″ or C-4″), 27.1 (C-1″), 33.7 (C-2′, 6′), 48.6 (C-1′), 55.3 (OMe-4), 61.6 (OMe-2), 72.8 (C-3″), 78.2 (C-2″), 96.3 (C-5), 110.2 (C-1), 114.1 (C-3), 124.8 (C-α), 132.3 (C-β), 158.7 (C-6), 160.3 (C-4), 160.9 (C-2), 167.4 (CO).

Methyltoddaliamide (**4**). Prisms (MeOH–EtOAc), mp 210–211°. UV λ_{max} nm (log ε): 220 (4.22), 240 (4.21), 260 (3.73), 300 (4.25), 320 (4.23). IR ν_{max} cm^{-1}: 3400 (OH), 3280 (NH), 1650 (NHCO). HRMS: $C_{23}H_{35}NO_6$. Found: 421.2470, calc. 421.2465. EIMS *m/z* (rel. int.): 421 $[M]^+$ (2), 322 (20), 250 (59), 219 (96), 207 (100), 161 (28), 56 (98). ^{1}H NMR (500 MHz, Me_2CO-d_6): δ 1.16 (1H, *m*, H-4a′), 1.18 (3H, *s*, Me), 1.19 (3H, *s*, Me), 1.26 (2H, *m*, H-3′a,5′a), 1.36 (2H, *m*, H-2′a,6′a), 1.62 (1H, *br d*, $J = 12.9$ Hz, H-4′b), 1.73 (2H, *m*, H-3′b,5′b), 1.91 (2H, *m*, H-2′b,6′b), 2.68 (1H, *dd*, $J = 10.2$, 13.6 Hz, H-1″a), 2.79 (1H, *dd*, $J = 2.6$, 13.6 Hz, H-1″b), 3.05 (1H, *d*, $J = 4.6$ Hz, OH-2″), 3.23 (3H, *s*, OMe-3″), 3.69 (1H, *m*, H-2″), 3.71 (3H, *s*, OMe-4), 3.77 (3H, *s*, OMe-2), 3.81 (1H, *m*, H-1′), 6.39 (1H, *s*, H-5), 6.92 (1H, *d*, $J = 15.9$ Hz, H-α), 7.03 (1H, *d*, $J = 7.4$ Hz, NH), 7.83 (1H, *d*, $J = 15.9$ Hz, H-β), 8.92 (1H, *s*, OH-6). ^{13}C NMR (100 MHz, Me_2CO-d_6): δ 21.1 (C-4″ or C-5″), 21.9 (C-5″ or C-4″), 26.2 (C-3′, 5′), 26.7 (C-4′), 26.9 (C-1″), 34.3 (C-2′, 6′), 49.3 (C-1′), 49.9 (OMe-3″), 56.3 (OMe-4), 62.1 (OMe-2), 77.0 (C-2″), 78.3 (C-3″), 96.9 (C-5), 110.3 (C-1), 114.9 (C-3), 123.8 (C-α), 132.3 (C-β), 158.2 (C-6), 160.9 (C-4), 161.3 (C-2), 167.3 (CO).

Toddasiatin (**5**). Yellow prisms, mp >300°. HRMS: $C_{30}H_{26}O_8$. Found: 514.1631, calc. 514.1625. EIMS *m/z* (rel. int.): 514 $[M]^-$ (100), 500 (36), 499 (99), 257 (15), 242 (47). UV λ_{max} nm (log ε): 226 (3.74), 260 (3.36), 270sh (3.34), 305sh (3.01), 360 (3.19). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.54 (6H, *s*, Mex2), 3.92 (3H, *s*, OMe), 5.77 (1H, *d*, $J = 9.9$ Hz, H-3′), 6.89 (1H, *s*, H-5), 6.93 (1H, *d*, $J = 9.9$ Hz, H-4′), 8.53 (1H, *s*, H-4).

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