Isoprenylated Flavonoids from the Roots and Rhizomes of *Sophora tonkinensis*

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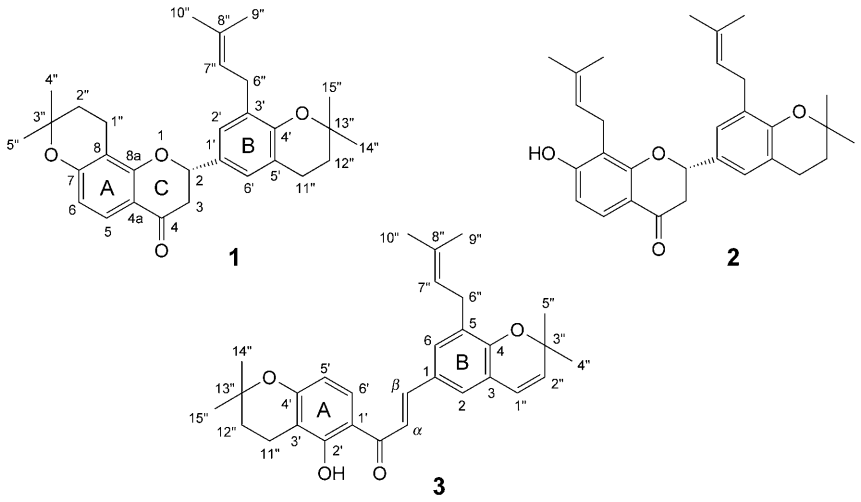
Two new flavanones, tonkinochromanes A (**1**) and B (**2**), and a new chalcone, tonkinochromane C (**3**), were isolated from the roots and rhizomes of *Sophora tonkinensis*. Their structures were elucidated by spectroscopic methods including 2D-NMR and MS experiments. Of the three isoprenyl substituents in these compounds on rings *A* and *B*, one to two were found to be ring-fused in the form of either dihydro- or regular 2,2-dimethyl-2*H*-pyran moieties.

Introduction. – *Sophora* species (Leguminosae), a rich source of isoprenyl- and lavandulyl-substituted flavonoids, have been investigated both phytochemically and biologically [1–5]. Some of the lavandulyl flavanones such as 2′-methoxykurarinone, kurarinone, sophoraflavanone G, and leachianone A exhibit potent antiproliferative effects on human myeloid leukemia HL-60 cells and human hepatocarcinoma HepG2 cells [6]; kurarinone also shows a significant growth-inhibitory effect on MCF-7/6 breast cancer cells [7].

In one of our previous papers, we reported the isolation of two new cytotoxic, isoprenylated flavonols from the roots of *S. flavescens* [8]. As a part of our continued search of cytotoxic phenolic compounds from *Sophora* plants, a phytochemical investigation on the flavonoid constituents of *S. tonkinensis* GAPNEP. (*S. subprostrata* CHUN et T. CHEN), a species widely distributed throughout Southwest China, was carried out. The roots and rhizomes of the title plant are commonly used as the traditional Chinese medicine '*Shandougen*' for the treatment of acute pharyngolaryngeal infections and sore throat [9], and pharmacological studies have shown that an isoprenylated flavanone isolated from this species, sophoranone, inhibits cell growth and induces apoptosis in various cell lines from human solid tumors and in human leukemia U937 cells [10].

In the present study, we report the isolation and structure elucidation of tonkinochromanes A–C (**1**–**3**), three new constituents obtained from the Et_2O-soluble fraction of the EtOH extract of the roots and rhizomes of *S. tonkinensis*.

Results and Discussion. – 1. *Structure Elucidation.* The Et_2O-soluble fraction of the EtOH extract of the roots and rhizomes of *S. tonkinensis* was subjected to repeated column chromatography and preparative TLC, which led to the isolation of tonkinochromanes A–C (**1**–**3**).



Tonkinochromane A (**1**) was obtained as a colorless oil, with $[\alpha]_D^{20} = -57.6$ ($c = 0.30$, MeOH). The quasi-molecular ion, $[M+Na]^+$, was detected by HR-ESI-MS at *m/z* 483.2511, consistent with the formula $C_{30}H_{36}O_4$. The IR spectrum showed absorption bands characteristic of a conjugated C=O group (1680 cm^{-1}) and an aromatic ring (1601, 1583, 1477 cm^{-1}). The UV spectrum exhibited maximum absorptions at 284 and 312 (sh) nm, which indicated a flavanone skeleton [11]. This was further supported by three reciprocally coupled H-atoms at δ(H) 5.40 (*dd*, $J = 12.9$, 2.8 Hz, 1 H), 2.98 (*dd*, $J = 16.7$, 12.9 Hz, 1 H), and 2.68 (*dd*, $J = 16.7$, 2.8 Hz, 1 H) in the ^{1}H-NMR spectrum of **1** (*Table 1*), which were assigned to H–C(2), H_a–C(3), and H_b–C(3), respectively, of the flavanone skeleton [11].

The ^{1}H-NMR spectrum of **1** showed the presence of a set of *ortho*-coupled aromatic H-atoms (δ(H) 7.59, 6.43 (2*d*, $J = 8.8$ Hz each, 2×1 H)), a set of *meta*-coupled ones (δ(H) 7.15, 7.10 (2 br. *s*, 2×1 H)), and a γ,γ-dimethylallyl (=3-methylbut-2-enyl) moiety attached to ring *B*, with signals at δ(H) 5.30 (*t*, $J = 7.4$ Hz, 1 H), 3.27 (*d*, $J = 7.4$ Hz, 2 H), 1.72 (*s*, 3 H), and 1.69 (*s*, 3 H) [12], together with two 3,4-dihydro-2,2-dimethyl-2*H*-pyran moieties fused to rings *A* and *B* of a flavanone, respectively, with signals at δ(H) 2.65 (*m*, 2 H), 1.80 (*m*, 2 H), 1.33 (*s*, 3 H), 1.31 (*s*, 3 H), and at δ(H) 2.80 (*t*, $J = 6.6$ Hz, 2 H), 1.81 (*t*, $J = 6.6$ Hz, 2 H), 1.33 (*s*, 6 H) [13] (*Table 1*). This assumption was confirmed by fragment ions at *m/z* 205 ($[A_1+H]^+$), 204 (A_1^+), and 256 (B_1^+) in the EI mass spectrum due to the *retro-Diels–Alder* cleavage of a flavanone *C*-ring [14]. The ^{13}C-NMR spectrum of **1** contained signals for a C=O group (δ(C) 190.7), three oxygenated aromatic C-atoms (161.6, 161.1, 152.6), as well as for C(2) (80.6) and C(3) (44.4) of the flavanone skeleton (*Table 2*).

The aromatic H-atom of **1** at δ(H) 7.59, which correlated with C(4) at δ(C) 190.7, C(7) at 161.1, and C(8a) at 161.6 in the HMBC spectrum (*Fig. 1*), could be assigned to H–C(5). Since it coupled with the *ortho*-positioned H–C(6) (δ(H) 6.43 (*d*, $J = 8.8$ Hz, 1 H)), the fusion site of the 2*H*-pyran moiety at ring *A* was established at C(7) and C(8). Further evidence for this deduction was provided by a HMBC experiment in which the CH_2 H-atoms at δ(H) 1.80 (CH_2(2″)) and 2.65 (CH_2(1″)) both correlated with C(8) at δ(C) 110.0; the H-atoms of CH_2(1″) also coupled with C(7) and C(8a) at δ(C) 161.1 and 161.6, respectively. The fusion site of the second 2*H*-pyran moiety at ring *B* was deduced to be at C(4′) and C(5′), based on correlations of the H-atoms at δ(H) 1.81 (CH_2(12″)) with C(5′) at δ(C) 121.3, and of the H-

Table 1. *^{1}H-NMR Data of* **1**–**3**. In (D_6)acetone; δ in ppm, J in Hz. Arbitrary atom numbering.

	1[a])	**2**[a])	**3**[b])
H–C(2)	5.40 (*dd*, *J* = 12.9, 2.8)	5.39 (*dd*, *J* = 12.8, 2.7)	7.46 (*d*, *J* = 2.0)
H_a–C(3)	2.98 (*dd*, *J* = 16.7, 12.9)	2.98 (*dd*, *J* = 16.7, 12.8)	–
H_b–C(3)	2.68 (*dd*, *J* = 16.7, 2.8)	2.70 (*dd*, *J* = 16.7, 2.7)	–
H–C(5)	7.59 (*d*, *J* = 8.8)	7.59 (*d*, *J* = 8.6)	–
H–C(6)	6.43 (*d*, *J* = 8.8)	6.62 (*d*, *J* = 8.6)	7.51 (*d*, *J* = 2.0)
7-OH	–	9.20 (br. *s*)	–
H–C(α)	–	–	7.76 (*AB*, *J* = 15.4)
H–C(β)	–	–	7.80 (*AB*, *J* = 15.4)
H–C(2′)	7.15 (br. *s*)	7.16 (br. *s*)	–
OH–C(2′)	–	–	14.13 (*s*)
H–C(5′)	–	–	6.33 (*d*, *J* = 8.9)
H–C(6′)	7.10 (br. *s*)	7.11 (br. *s*)	7.94 (*d*, *J* = 8.9)
CH_2(1″) or H–C(1″)	2.65 (*m*)	3.35 (*d*, *J* = 7.1)	6.45 (*d*, *J* = 9.8)
CH_2(2″) or H–C(2″)	1.80 (*m*)	5.26 (*t*, *J* = 7.1)	5.81 (*d*, *J* = 9.8)
Me(4″)	1.31 (*s*)	1.64 (*s*)	1.44 (*s*)
Me(5″)	1.33 (*s*)	1.66 (*s*)	1.44 (*s*)
CH_2(6″)	3.27 (*d*, *J* = 7.4)	3.28 (*d*, *J* = 7.3)	3.30 (*d*, *J* = 7.4)
H–C(7″)	5.30 (*t*, *J* = 7.4)	5.31 (*t*, *J* = 7.3)	5.30 (br. *t*, *J* = 7.4)
Me(9″)	1.72 (*s*)	1.73 (*s*)	1.71 (*s*)
Me(10″)	1.69 (*s*)	1.70 (*s*)	1.76 (*s*)
CH_2(11″)	2.80 (*t*, *J* = 6.6)	2.82 (*t*, *J* = 6.7)	2.67 (*t*, *J* = 6.8)
CH_2(12″)	1.81 (*t*, *J* = 6.6)	1.84 (*t*, *J* = 6.7)	1.84 (*t*, *J* = 6.8)
Me(14″)	1.33 (*s*)	1.34 (*s*)	1.34 (*s*)
Me(15″)	1.33 (*s*)	1.34 (*s*)	1.34 (*s*)

[a]) At 500 MHz. [b]) At 400 MHz.

atoms at δ(H) 2.80 (CH_2(11″)) with C(4′) at δ(C) 152.6, C(5′) at 121.3, and C(6′) at 126.1, which contained a H-atom at δ(H) 7.10 (H–C(6′)) correlating with C(2) at δ(C) 80.6 in the HMBC spectrum. In addition, the γ,γ-dimethylallyl group at ring *B* was deduced to be adjacent to C(3′), based on the HMBC cross-peak of H–C(7″) at δ(H) 5.30 with C(3′) at δ(C) 130.3, and due to cross-peaks of the H-atoms of CH_2(6″) at δ(H) 3.27 with C(4′) at δ(C) 152.6, C(3′) at 130.3, and C(2′) at 126.3, bearing H–C(2′) at δ(H) 7.15, which correlated with C(2) at δ(C) 80.6 (*Fig. 1*). Thus, with the aid of HMBC and HMQC experiments, all ^{1}H- and ^{13}C-NMR signals were fully assigned.

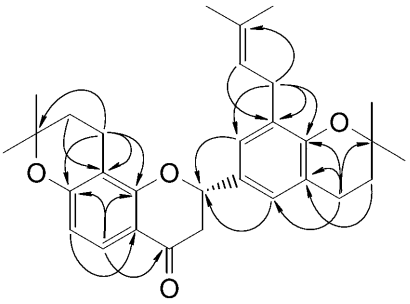


Fig. 1. *Selected HMBC correlations of* **1**

Table 2. *^{13}C-NMR Data of* **1**–**3**. In (D_6)acetone; δ in ppm. Arbitrary atom numbering.

	1[a])	**2**[a])	**3**[b])		**1**	**2**	**3**
C(1)	–	–	128.3	C(5′)	121.3	121.7	109.7
C(2)	80.6	80.9	125.7	C(6′)	126.1	126.5	129.8
C(3)	44.4	45.0	122.3	C(1″)	17.5	23.2	122.7
C(4)	190.7	191.4	154.1	C(2″)	32.3	123.6	132.2
C(4a)	114.7	115.9	–	C(3″)	76.0	132.0	77.9
C(5)	126.0	126.67	130.5	C(4″)	26.5	26.3	28.3
C(6)	112.2	110.8	131.7	C(5″)	27.1	18.4	28.3
C=O	–	–	192.9	C(6″)	29.4	29.8	29.0
C(α)	–	–	118.8	C(7″)	123.8	124.3	123.4
C(β)	–	–	145.1	C(8″)	132.1	132.4	132.5
C(7)	161.1	162.6	–	C(9″)	17.9	18.3	25.9
C(8)	110.0	116.9	–	C(10″)	25.9	26.3	18.0
C(8a)	161.6	162.4	–	C(11″)	23.3	23.7	17.0
C(1′)	130.9	131.5	113.6	C(12″)	33.3	33.7	32.3
C(2′)	126.3	126.70	165.0	C(13″)	75.0	75.3	76.4
C(3′)	130.3	130.7	109.9	C(14″)	27.2	27.64	26.9
C(4′)	152.6	152.9	161.6	C(15″)	27.3	27.60	26.9

[a]) At 125 MHz. [b]) At 100 MHz.

The absolute configuration at C(2) of **1** was determined as (*S*) from the circular-dichroism (CD) spectrum, which showed a positive *Cotton* effect at 333 nm, and a negative one at 304 nm [4]. Thus, compound **1** was identified as (2*S*)-2-[3,4-dihydro-2,2-dimethyl-8-(3-methylbut-2-enyl)-2*H*-1-benzopyran-6-yl]-2,3,9,10-tetrahydro-8,8-dimethyl-4*H*,8*H*-benzo[1,2-*b*:3,4-*b*′]dipyran-4-one, and named tonkinochromane A.

Tonkinochromane B (**2**) was obtained in the form of colorless needles melting at 190–192°, with $[\alpha]_D^{20} = -22.1$ ($c = 0.20$, MeOH). The compound had the molecular formula $C_{30}H_{36}O_4$, as established by HR-ESI-MS ($[M+H]^+$ at *m/z* 461.2691). Its UV and IR spectra were similar to those of **1**, except for an additional IR absorption band at 3286 cm^{-1} characteristic of an OH group.

Comparison of the ^{1}H-NMR data of **2** with those of **1** revealed that the chemical-shift values and splitting patterns of **2** agreed well with those of **1**, except that the signals for a 3,4-dihydro-2,2-dimethyl-2*H*-pyran moiety at ring *A* of **1** (δ(H) 2.65 (*m*, 2 H), 1.80 (*m*, 2 H), 1.33 (*s*, 3 H), 1.31 (*s*, 3 H)) were replaced by the signals for a γ,γ-dimethylallyl group (δ(H) 5.26 (*t*, $J = 7.1$ Hz, 1 H), 3.35 (*d*, $J = 7.1$ Hz, 2 H), 1.66 (*s*, 3 H), 1.64 (*s*, 3 H)) and an OH group (δ(H) 9.20 (br. *s*, 1 H)) in **2**, and that H–C(6) of the latter was shifted downfield by 0.19 ppm (*Table 1*). The ^{13}C-NMR data of **2** were also identical to those of **1**, except for the corresponding replacement of the carbon signals for the dihydropyran moiety at ring *A* of **1** (δ(C) 17.5, 32.3, 76.0, 26.5, and 27.1) by the signals for a γ,γ-dimethylallyl chain (δ(C) 23.2, 123.6, 132.0, 26.3, and 18.4) in **2**, and for the downfield shift of C(8) ($\Delta\delta$(C) = +6.9 ppm; *Table 2*).

From the above data, it could be inferred that **2** contained an isoprenyl side chain and a neighboring OH group at ring *A*, rather than the dihydropyran moiety in **1**. This was also supported by the fragment ions at *m/z* 205 ($[A_1+H]^+$), 204 (A_1^+), and 256 (B_1^+) in the EI mass spectrum due to the *retro-Diels–Alder* cleavage of a flavanone *C*-ring, as well as the ion at *m/z* 149 ($[A_1-C_4H_7]^+$) resulting from the cleavage of an isoprenyl side chain from A_1 [14]. The proposed structure of **2** was further confirmed by HMBC and HMQC experiments.

The absolute configuration of **2** at C(2) was determined as (*S*) from the CD spectrum, which showed a positive *Cotton* effect at 334 nm, and a negative one at 303 nm [4]. Thus, compound **2** was deduced to correspond to (2*S*)-3′,4′-dihydro-7-hydroxy-2′,2′-dimethyl-8,8′-bis(3-methylbut-2-enyl)-[2,6′-bi-2*H*-1-benzopyran]-4(3*H*)-one, and named tonkinochromane B.

Tonkinochromane C (**3**), obtained as a yellow oil, had the molecular formula $C_{30}H_{34}O_4$, as inferred from the quasi-molecular ion at *m/z* 481.2352 in its HR-ESI mass spectrum ($[M+Na]^+$). The compound tested positive to the $FeCl_3$ reagent, which indicated a phenolic moiety. Its IR spectrum exhibited absorption bands characteristic of an OH group (3441 cm^{-1}), an α,β-unsaturated C=O group (1628 cm^{-1}), and an aromatic ring (1588, 1563, 1486 cm^{-1}). In the UV spectrum, the maxima at 383, 295, and 230 (sh) nm, as well as a pair of *AB*-type *quartet*s centered at δ(H) 7.76 and 7.80 ($J=15.4$ Hz, 1 H each) in the ^{1}H-NMR spectrum suggested the presence of a chalcone skeleton [11].

The ^{1}H-NMR spectrum of **3** exhibited signals for a 2,2-dimethyl-2*H*-pyran ring (δ(H) 6.45 (*d*, $J=9.8$ Hz, 1 H), 5.81 (*d*, $J=9.8$ Hz, 1 H), 1.44 (*s*, 6 H)), a 3,4-dihydro-2,2-dimethyl-2*H*-pyran moiety (δ(H) 2.67 (*t*, $J=6.8$ Hz, 2 H), 1.84 (*t*, $J=6.8$ Hz, 2 H), 1.34 (*s*, 6 H)), and a γ,γ-dimethylallyl side chain (δ(H) 5.30 (br. *t*, $J=7.4$ Hz, 1 H), 3.30 (*d*, $J=7.4$ Hz, 2 H), 1.76 (*s*, 3 H), 1.71 (*s*, 3 H)) similar to those in **1** (*Table 1*). Further, a chelated OH group was observed (δ(H) 14.13 (*s*, 1 H)), together with a pair of *ortho*-coupled aromatic H-atoms (δ(H) 7.94 (*d*, $J=8.9$ Hz, 1 H), 6.33 (*d*, $J=8.9$ Hz, 1 H)), and a set of *meta*-coupled ones (δ(H) 7.51 (*d*, $J=2.0$ Hz, 1 H), 7.46 (*d*, $J=2.0$ Hz, 1 H)) (*Table 1*). The resonance at δ(H) 7.94 (*d*, $J=8.9$ Hz, 1 H) could be assigned to H–C(6′) due to its downfield chemical shift resulting from the deshielding effect of the neighboring C=O group, and its correlations with the C-atoms at δ(C) 192.9 (C=O), 165.0 (C(2′)), and 161.6 (C(4′)) in the HMBC spectrum (*Fig. 2*). Since it coupled with the *ortho*-positioned H–C(5′) (δ(H) 6.33 (*d*, $J=8.9$ Hz)), the 3,4-dihydro-2,2-dimethyl-2*H*-pyran moiety at ring *A* was determined to be attached to C(3′) and C(4′), as further corroborated by the following HMBC correlations: CH_2(12″) at δ(H) 1.84 with C(3′) at δ(C) 109.9; CH_2(11″) at δ(H) 2.67 with C(2′) at δ(C) 165.0, C(3′) at 109.9, and C(4′) at 161.6; 2′-OH at δ(H) 14.13 with C(1′) at δ(C) 113.6, C(2′) at 165.0, and C(3′) at 109.9 (*Fig. 2*).

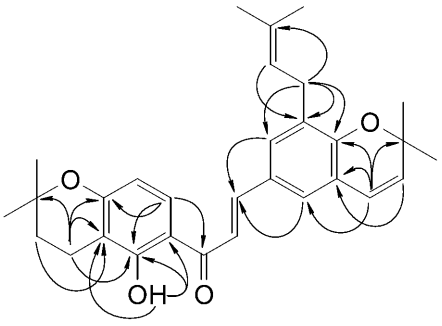


Fig. 2. *Selected HMBC correlations of* **3**

As to ring *B*, the two *meta*-coupled aromatic H-atoms (δ(H) 7.51, 7.46 (2*d*, $J=2.0$ Hz each, 2×1 H)) both correlated with C(β) at δ(C) 145.1 in the HMBC spectrum, indicating that the substituents were located at C(3), C(4), and C(5). The γ,γ-dimethylallyl chain was determined to be connected to C(5), and the 2,2-dimethyl-2*H*-pyran ring was fused to C(3) and C(4), respectively, as inferred from the following HMBC cross-peaks: H–C(6) at δ(H) 7.51 with C(6″) at δ(C) 29.0, C(4) at 154.1, and C(β) at 145.1; CH_2(6″) at δ(H) 3.30 with C(4) at δ(C) 154.1, C(5) at 130.5, and C(6) at 131.7; H–C(2) at δ(H) 7.46 with

C(1″) at δ(C) 122.7, C(4) at 154.1, and C(β) at 145.1; H–C(1″) at δ(H) 6.45 with C(2) at δ(C) 125.7, C(3) at 122.3, and C(4) at 154.1 (*Fig. 2*). With the aid of HMBC and HMQC experiments, all ^{1}H- and ^{13}C-NMR signals could be fully assigned.

From the above data, compound **3** was identified as (2*E*)-1-(3,4-dihydro-5-hydroxy-2,2-dimethyl-2*H*-1-ben zopyran-6-yl)-3-[2,2-dimethyl-8-(3-methylbut-2-enyl)-2*H*-1-benzopyran-6-yl]prop-2-en-1-one, and named tonkinochromane C.

2. *Structure–Activity Relationship.* The two isolated flavanones **1** and **2**[1]) were tested *in vitro* against a panel of human tumor cell lines, including human lung carcinoma (A549), ovarian carcinoma (1A9), breast adenocarcinoma (MCF-7), epidermoid carcinoma of the nasopharynx (KB) and its drug-resistant variant (KB-Vin), but none of them showed potent cytotoxic activity.

In our previous studies [8][15], six flavonoids, isoprenylated in the form of 3,4-dihydro-2,2-dimethyl-2*H*-pyran moieties, had been isolated from *S. flavescens*. Two of them, flavenochromanes B and C, are strongly cytotoxic against the A549, 1A9, MCF-7, KB, and KB-Vin cell lines, with IC_{50} values in the range of 1.0–6.9 μM [8]. Based on the analysis of the cytotoxic potencies and the structural characteristics of these tested compounds from *S. tonkinensis* and *S. flavescens*, it was found that the flavonols with two hydrophobic groups at ring *A* and a hydrophilic group at ring *B* are the most-cytotoxic ones, while the flavanonols, flavanones, and flavanols with hydrophobic groups at both rings *A* and *B* are almost inactive. This indicates that the distribution of hydrophobic and hydrophilic groups at different rings of the flavonoid skeleton might be important for cytotoxic activity. This structure–activity relationship (SAR) is quite similar to those reported previously. There, the cytotoxic flavones and xanthones were found to have the most-hydrophobic groups at one domain (ring *A*), and the most-hydrophilic ones at another (ring *B*) [16–18]. However, as to the apoptogenic isoprenyl- or lavandulyl-substituted flavonoids such as sophoranone, sophoraflavanone G, and leachianone A, they have hydrophobic and hydrophilic groups at both rings *A* and *B* [6][10]. Therefore, further investigations are necessary to fully understand the structural determinants for cytotoxic activity of these isoprenylated flavonoids.

Experimental Part

General. Column chromatography (CC): silica gel *H* (200–300 mesh; *Qingdao*, China). Anal. (0.25 mm) and prep. (0.50 mm) TLC: precoated silica-gel plates GF_{254} (10–40 μm; *Yantai*, China); detection by UV light and visualization by spraying with 10% aq. H_2SO_4 followed by heating. Melting points (m.p.): *XT-4* micro-melting-point apparatus; uncorrected. UV Spectra: *Shimadzu UV-260* UV/VIS recording spectrophotometer; λ_{max} (log ε) in nm. Optical rotation: *Jasco P-1020* digital polarimeter. CD Spectra: *JASCO J-715* spectropolarimeter; λ ([θ]) in nm. IR Spectra: *Avatar 360-FT-IR* spectrophotometer; KBr pellets or CH_2Cl_2 soln.; in cm^{-1}. NMR Spectra: *Bruker DRX-400* or *-500* spectrometers, at 400 or 500 (^{1}H), and at 100 or 125 MHz (^{13}C), resp., in (D_6)acetone soln. at r.t.; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. EI-MS: *Hewlett-Packard 5989A* mass spectrometer; in *m/z* (rel. %). HR-ESI-MS: *AB-QSTAR-Pulsar* mass spectrometer.

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[1]) Tonkinochromane C (**3**) could not be tested due to the limited amount of sample available.

Plant Material. The roots and rhizomes of *Sophora tonkinensis* GAPNEP. were purchased from *Huayu Materia Medica Co., Ltd.*, Shanghai, in March 2000. A voucher specimen (SDG-SH-0003) was deposited at the Herbarium of Materia Medica, Department of Pharmacognosy, School of Pharmacy, Fudan University, Shanghai, P. R. China.

Extraction and Isolation. The pulverized roots and rhizomes of *S. tonkinensis* (9 kg) were extracted with 1% (*v*/*v*) aq. H_2SO_4 soln. (4×), which afforded the total-alkaloid extract (120 g). The remaining residue was air-dried, and extracted with 95% EtOH (6×12 l) at r.t. The evaporated EtOH extract (1.5 kg) was suspended in H_2O (2.5 l) and extracted with Et_2O (6×3 l). The Et_2O-soluble fraction (220 g) was subjected to CC (SiO_2; petroleum ether (PE; b.p. 60–90°)/AcOEt 100:1, 50:1, 30:1, 20:1, 10:1, 5:1, 2:1, 1:1, and 0:1): *Fractions 1–9*. *Fr. 2* (10.05 g) was subjected to CC (SiO_2, PE/Me_2CO 80:1): *Fr. 2A–E*. *Fr. 2A* (1.25 g) was subjected to CC (SiO_2, PE/AcOEt 50:1) and then to prep. TLC (SiO_2 (*GF_{254}*); PE/Me_2CO 100:3), which afforded **3** (6 mg). *Fr. 2B* (2.50 g) was subjected to CC (SiO_2; PE/Et_2O 95:5) and then to prep. TLC (SiO_2; PE/AcOEt 92:8), which afforded **1** (30 mg). *Fr. 5* (2.16 g) was subjected to CC (SiO_2; PE/$CHCl_3$/Me_2CO 50:50:1), which gave **2** (35 mg).

Tonkinochromane A (=*(2*S*)-2-[3,4-Dihydro-2,2-dimethyl-8-(3-methylbut-2-enyl)-2*H*-1-benzopyran-6-yl]-2,3,9,10-tetrahydro-8,8-dimethyl-4*H,*8*H*-benzo[1,2*-b*:3,4*-b′*]dipyran-4-one*; **1**). Colorless oil. UV (MeOH): 312 (sh, 3.93), 284 (4.27), 230 (sh, 4.34), 224 (4.39). $[\alpha]_D^{20} = -57.6$ ($c = 0.30$, MeOH). CD (MeOH): 333 (+22080), 304 (−40395), 243 (+18796), 222 (+5477). IR (CH_2Cl_2): 2975, 2929, 2853, 1680, 1601, 1583, 1477, 1435, 1369, 1330, 1267, 1231, 1208, 1158, 1121, 1100, 1068, 1056, 943, 880, 814, 784, 737. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. EI-MS: 460 (22, M^+), 256 (29), 244 (19), 243 (100), 205 (15), 204 (6), 203 (25), 202 (16), 201 (57), 189 (13), 185 (48), 176 (12), 175 (10), 161 (30), 157 (23), 149 (78), 145 (54), 141 (12), 129 (13), 128 (16), 117 (13), 115 (27), 91 (21), 69 (21), 55 (20), 43 (30), 41 (29). HR-ESI-MS: 483.2511 ($[M+Na]^+$, $C_{30}H_{36}NaO_4^+$; calc. 483.2511).

Tonkinochromane B (=*(2*S*)-3′,4′-Dihydro-7-hydroxy-2′,2′-dimethyl-8,8′-bis(3-methylbut-2-enyl)-[2,6′-bi-2*H*-1-benzopyran]-4(3*H*)-one*; **2**). Colorless fine needles. M.p. 190–192° (petroleum ether/Me_2CO 5:1). UV (MeOH): 314 (sh, 3.69), 286 (4.09), 234 (sh, 4.28), 213 (4.43). $[\alpha]_D^{20} = -22.1$ ($c = 0.20$, MeOH). CD (MeOH): 334 (+12166), 303 (−20965), 244 (+6415), 208 (+21876). IR (KBr): 3286, 2974, 2923, 2849, 1661, 1597, 1582, 1479, 1444, 1368, 1339, 1282, 1229, 1208, 1159, 1046, 945, 892, 877, 817, 776, 718. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. EI-MS: 461 (3), 460 (2, M^+), 319 (3), 256 (1), 239 (7), 205 (1), 204 (3), 203 (11), 201 (14), 189 (14), 187 (14), 185 (33), 177 (3), 176 (13), 175 (10), 161 (36), 160 (16), 149 (78), 141 (13), 131 (13), 129 (11), 128 (18), 117 (10), 116 (10), 115 (32), 91 (19), 77 (12), 69 (24), 56 (18), 55 (37), 53 (17), 43 (100), 42 (38), 41 (64). HR-ESI-MS: 461.2691 ($[M+H]^+$, $C_{30}H_{37}O_4^+$; calc. 461.2686).

Tonkinochromane C (=*(2*E*)-1-(3,4-Dihydro-5-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-yl)-3-[2,2-dimethyl-8-(3-methylbut-2-enyl)-2*H*-1-benzopyran-6-yl]prop-2-en-1-one*; **3**). Yellow oil. UV (MeOH): 383 (4.68), 295 (4.28), 230 (sh, 4.51), 209 (4.65). IR (CH_2Cl_2): 3441, 3053, 2978, 2927, 2849, 1628, 1588, 1563, 1486, 1466, 1434, 1363, 1266, 1221, 1154, 1109, 1049, 980, 919, 895, 881, 795, 739, 704. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. EI-MS: 458 (19, M^+), 444 (24), 443 (71), 281 (4), 239 (23), 205 (5), 165 (13), 149 (35), 128 (11), 115 (12), 97 (10), 95 (10), 91 (15), 83 (17), 81 (18), 79 (12), 77 (14), 72 (31), 71 (12), 69 (37), 67 (23), 60 (12), 59 (57), 57 (47), 56 (16), 55 (73), 54 (13), 53 (13), 44 (40), 43 (86), 42 (18), 41 (100), 39 (19). HR-ESI-MS: 481.2352 ($[M+Na]^+$, $C_{30}H_{34}NaO_4^+$; calc. 481.2355).

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