Xanthine Oxidase Inhibitors from the Heartwood of Vietnamese *Caesalpinia sappan*

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From the MeOH extract of Vietnamese *Caesalpinia sappan*, a novel biogenetically exclusive benzindenopyran, with a new carbon framework, neoprotosappanin (1), and a new compound, protosappanin A dimethyl acetal (3), were isolated together with protosappanin E-2 (2), neosappanone A (4), and 13 previously reported phenolic compounds (5—17). Their structures were elucidated on the basis of spectroscopic data. Compounds 1—4, 7, 13, and 15—17 showed significant xanthine oxidase inhibitory activity in a concentration-dependent manner, and sappanchalcone (17) showed the most potent activity with an IC_{50} value of 3.9 μM, comparable to that of positive control allopurinol (IC_{50}, 2.5 μM). The kinetic study of these inhibitors indicated that they are competitive inhibitors, the same as allopurinol, except for 1 and 16 which are noncompetitive inhibitors.

Key words *Caesalpinia sappan*; benzindenopyran; xanthine oxidase inhibition

Xanthine oxidase (XO) is a key enzyme that catalyses the oxidation of xanthine and hypoxanthine into uric acid,[1] and plays a vital role in producing hyperuricemia and gout. Allopurinol is a clinically used XO inhibitor in the treatment of gout, which blocks the terminal step in uric acid biosynthesis can lower the plasma uric acid concentration.[2] However, due to unwanted side effects of allopurinol, such as hepatitis, nephropathy, and allergic reactions, new alternatives with increased therapeutic activity and less side effects are desired. Moreover, superoxide anion radicals generated by XO are involved in various pathological states such as hepatitis, inflammation, aging, carcinogenesis, and ischemia-reperfusion.[3] Thus, the search for novel XO inhibitors would be beneficial not only to treat gout but also to combat various other diseases.

The heartwood of *Caesalpinia sappan* L. (Caesalpiniaceae) has been used in Vietnamese traditional medicine for the treatment of rheumatism and inflammatory diseases and as an emmenagogue and homeostatic agent.[4] Our preliminary screening study revealed that the methanolic extract of the heartwood of *C. sappan* exhibited significant XO inhibitory activity with an IC_{50} value of 14.2 μg/ml.[5] Therefore, we carried out fractionation of the MeOH extract and isolated a dibenzoxocin possessing a novel carbon skeleton named neosappanone A (**4**), which was reported in our preliminary communication.[6] Further investigation on this active MeOH extract led to the isolation of 16 additional phenolic compounds including a new benzindenopyran, neoprotosappanin (**1**) having a novel carbon framework, protosappanin E-2 (**2**), and protosappanin A dimethyl acetal (**3**). In this paper, we report the isolation and structure elucidation of these compounds by spectroscopic techniques, together with the XO inhibitory activity of the isolated compounds.

Results and Discussion

The dried heartwood of *C. sappan* was extracted with refluxing MeOH, and the MeOH extract was fractionated with EtOAc to yield EtOAc-soluble fraction. The EtOAc-soluble fraction showed XO inhibitory activity with an IC_{50} value of 12.8 μg/ml. Further separation and purification of this fraction led to the isolation of new compounds **1** and **3**, together with neosappanone A (**4**).[6] The known compounds were identified by analysis of their spectroscopic data and comparison with literature data to be protosappanin E-2 (**2**),[7] protosappanin B (**5**),[8] protosappanin C dimethyl acetal (**6**),[9] protosappanin A (**7**),[10] brazilin (**8**),[11] 3′-*O*-methylbrazilin (**9**),[12] 3′-deoxysappanol (**10**),[13,14] 3′-deoxy-4-*O*-methylsappanol (**11**),[15] 4-*O*-methylsappanol (**12**),[12,16] sappanol (**13**),[15] 4-*O*-methylepisappanol (**14**),[12,16] sappanone B (**15**),[12,15] 3-deoxysappanone B (**16**),[12,16] and sappanchalcone (**17**).[17]

Neoprotosappanin (**1**) showed a quasimolecular ion at *m*/*z* 571.1586 $[M+H]^+$, corresponding to the molecular formula $C_{32}H_{26}O_{10}$ in high resolution fast atom bombardment mass spectrometry (HR-FAB-MS). The IR spectrum of **1** displayed the absorbance of hydroxyl (3300 cm^{-1}) and phenyl (1610, 1450 cm^{-1}) groups. The ^{1}H-NMR spectrum of **1** displayed signals due to two oxymethylenes, an aliphatic methylene, three aliphatic methines, six singlets of three 1,2,4,5-tetrasubstituted phenyl protons, and three aromatic protons of typical ABX system (Table 1). The ^{13}C-NMR spectrum showed 32 carbon signals including two oxygenated quaternary carbons, two oxymethylene carbons, three aliphatic methine carbons, an aliphatic methylene carbon, and 24 aromatic carbons (Table 1). These ^{1}H- and ^{13}C-NMR signals appeared as related pairs, indicating that **1** has a dimeric carbon skeleton with two similar units (units I and II).

Unit I of neoprotosappanin (**1**) displayed the typical signals of an ABX system [δ 7.20 (d, J=8.5 Hz, H-1), 6.44 (dd, J=8.5, 2.4 Hz, H-2), 6.30 (d, J=2.4 Hz, H-4)] and two aromatic singlets [δ 6.58 (H-8), 6.89 (H-11)], together with two aliphatic methines [δ 4.49 (H-7), 4.02 (H-11b)], and an oxymethylenes [δ 4.01 and 3.97 (d, J=11.2 Hz, H-6)] protons in the ^{1}H-NMR spectrum (Table 1). On the other hand, the ^{13}C-NMR data for unit I indicated the presence of an oxygenated quaternary carbons [δ 80.5 (C-6a)], an oxymethylene carbon [δ 71.1 (C-6)], two aliphatic methine carbons [δ 49.2 (C-7), 50.3 (C-11b)], together with five aromatic carbons [δ 131.9 (C-1), 110.0 (C-2), 104.2 (C-4), 114.1 (C-8), 112.8 (C-11)], three aromatic quaternary carbons [δ 115.9 (C-11c), 137.9 (C-7a), 135.2 (C-11a)], and four oxygenated aromatic carbons [δ 157.8 (C-3), 155.7 (C-4a), 145.6 (C-9), 146.0 (C-10)]. These data were similar to those of brazilin

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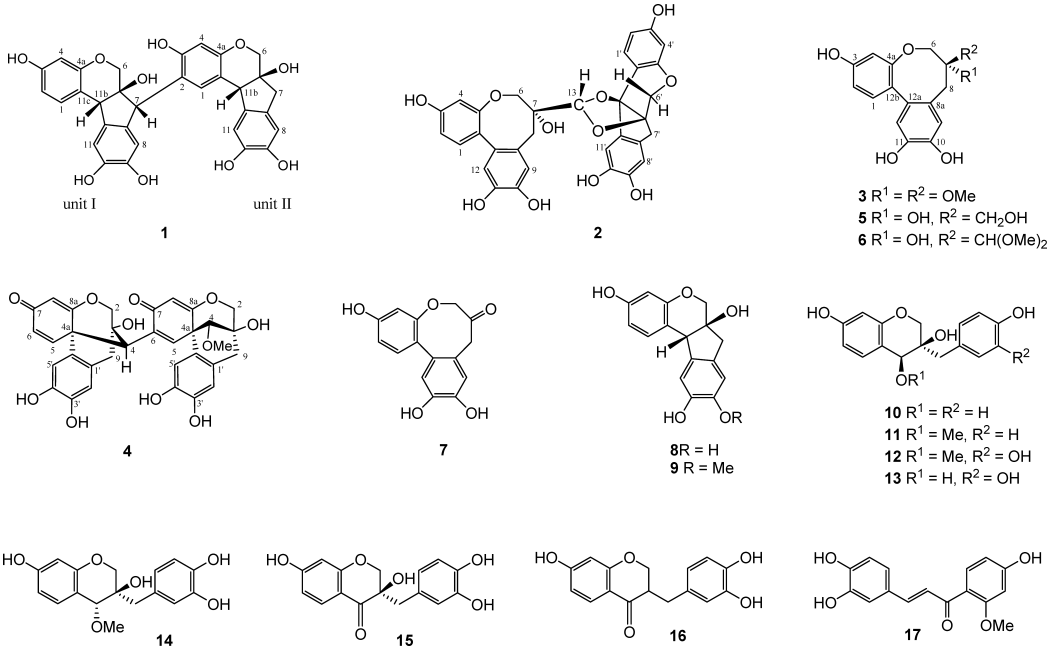

Chart 1. Structures of Isolated Compounds from the Heartwood of *C. sappan*

Table 1. ^{1}H- and ^{13}C-NMR Data for Compound **1** in CD_3OD

Unit	Position	δ_H	δ_C
I	1	7.20 d (8.5)	131.9
	2	6.44 dd (8.5, 2.4)	110.0
	3		157.8
	4	6.30 d (2.4)	104.2
	4a		155.7
	6	4.01 d (11.2); 3.97 d (11.2)	71.1
	6a		80.5
	7	4.49 s	49.2
	7a		137.9
	8	6.58 s	114.1
	9		145.6
	10		146.0
	11	6.89 s	112.8
	11a		135.2
	11b	4.02 s	50.3
	11c		115.9
II	1	6.73 s	133.8
	2		120.2
	3		156.3
	4	6.37 s	104.2
	4a		154.5
	6	3.89 d (11.2); 3.67 d (11.2)	70.9
	6a		78.0
	7	2.99 d (15.9); 2.73 d (15.9)	42.9
	7a		137.4
	8	6.56 s	112.6
	9		144.9
	10		145.5
	11	6.44 s	112.8
	11a		131.5
	11b	3.86 s	51.1
	11c		115.3

J values in parentheses.

(**8**),[11] isolated from the same extract, except for the disappearance of signals due to an aliphatic methylene protons in **8**, [H_2-7: δ 3.01 (d, J=15.4 Hz), 2.76 (d, J=15.4 Hz)] and appearance of an aliphatic methine proton. Further heteronuclear multiple bond correlation (HMBC) spectral analysis (Fig. 1) led to the confirmation of monomeric unit I.

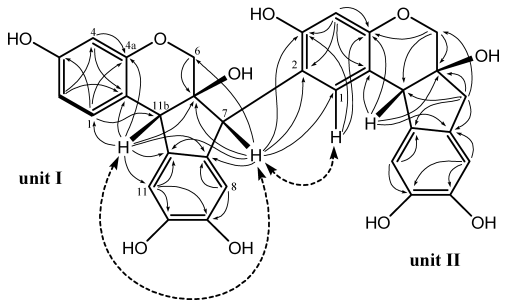

Fig. 1. Connectivities (Bold Line) Deduced by the COSY Spectrum, Significant HMBC Correlations (Arrows), and ROESY Correlations

Unit II, on the other hand, also displayed the signals similar to those of **8** in the ^{1}H- and ^{13}C-NMR spectra, except for the disappearance of signals due to an ABX system in **8**, and appearance of two singlets [δ 6.73 (H-1), 6.37 (H-4)] in **1**. This suggested that the two singlets in unit II should be *para* to each other. Thus, the linkage between units I and II should be *via* C-7 in unit I and C-2 in unit II, which was confirmed by the HMBC correlations of H-7 in unit I with C-1, C-2, and C-3 in unit II (Fig. 1). The relative stereochemistry at C-6a and C-11b in each brazilin monomer unit should be the same as those of **8** by comparision of its spectroscopic data and literature data,[11,18] as well as biogenetic consideration. Further, the rotating frame nuclear Overhauser effect spectroscopy (ROESY) correlations between H-7 and H-11b in unit I indicated that H-7 to be β-oriented (Fig. 1). Thus, the structure of neoprotosappanin was concluded as **1**.

Protosappanin E-2 (**2**) was obtained as a yellow amorphous solid. Its molecular formula was determined by HR-FAB-MS to be $C_{32}H_{26}O_{11}$. The IR spectrum of **2** showed the absorptions due to hydroxyl (3300 cm^{-1}) and phenyl (1600, 1450 cm^{-1}) groups. The ^{1}H-NMR spectrum of **2** displayed signals due to two oxymethylenes, two aliphatic methylene,

Table 2. ^{1}H- and ^{13}C-NMR Data for Compounds **2** and **3** in CD_3OD

Position	**2**		**3**	
	δ_H	δ_C	δ_H	δ_C
1	6.97 d (8.5)	113.0	6.92 d (8.8)	134.4
2	6.52 dd (8.5, 2.2)	111.4	6.46 dd (8.8, 2.4)	110.7
3		159.3		158.5
4	6.27 d (2.2)	104.0	6.37 d (2.4)	107.0
4a		159.8		158.1
6	4.15 d (11.5); 4.02 d (11.5)	69.1	4.24 d (12.9); 3.68 d (12.9)	68.5
7		89.5		100.5
8	3.18 d (16.3); 2.96 d (16.3)	40.2	2.92 d (13.2); 2.55 d (13.2)	37.4
8a		135.0		127.2
9	6.58 s	112.5	6.64 s	117.5
10		146.1		144.6
11		148.0		144.8
12	6.98 s	132.1	6.71 s	118.6
12a		132.7		132.5
12b		116.9		121.0
13	5.0 s	108.2		
1′	7.58 d (8.5)	130.5		
2′	6.52 dd (8.5, 2.2)	112.1		
3′		159.0		
4′	6.28 d (2.2)	108.8		
4a′		156.9		
6′	4.10 d (11.2); 3.75 d (11.2)	78.1		
6a′		73.1		
7′	2.52 d (14.2); 2.47 d (14.2)	38.3		
7a′		127.2		
8′	6.64 s	120.5		
9′		144.8		
10′		145.0		
11′	6.64 s	117.3		
11a′		132.2		
11b′		88.9		
11c′		125.7		
7-OCH_3×2			3.34 s	49.2

J values in parentheses.

an acetal methine, four singlets of two 1,2,4,5-tetrasubstituted benzene rings, and six aromatic protons consisting of two ABX system (Table 2). The ^{13}C-NMR spectrum showed 32 carbon signals including three oxygenated quaternary carbons and 24 aromatic carbons (Table 2). Analysis of the ^{1}H-NMR spectrum indicated two distinct groups of signals ascribable to protosappanin B (unit I) and brazilin (unit II) moieties. However, it showed slight difference in the ^{1}H-NMR spectral data due to presence of an acetal methine at δ 5.0 instead of an oxymethylene at δ 3.53 in protosappanin B (**5**), and the disappearance of signal due to an aliphatic methine in brazilin (**8**), respectively. Furthermore, its ^{13}C-NMR spectrum displayed one more acetal carbon instead of an oxymethylene carbon in **5**, and oxygenated quaternary carbon instead of an aliphatic methine carbon in **8**. These data indicated that the protosappanin B moiety should be linked to brazilin moiety *via* the acetal carbon C-13 (δ 108.2). Further HMBC correlations (Fig. 2) of H-13 (δ 5.0) (unit I) with C-6a′ (δ 73.1) and C-11b′ (δ 88.9) (unit II) led to the complete assignment of the linkage between unit I and unit II. In the ROESY experiment, the significant correlations H-13/H-6′β and H-13/H-6β suggested that they orientate on the same side. Analysis of these ROESY correlations by means of the Drieding stereomodel indicated that the hydroxyl group at C-7 should be α-oriented. Literature survey of the compound with the structure indicated that **2** should be protosappanin

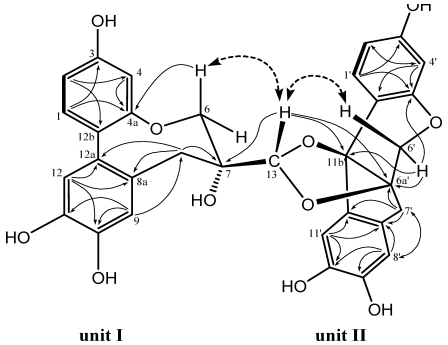

Fig. 2. Connectivities (Bold Line) Deduced by the COSY Spectrum, Significant HMBC Correlations (Arrows), and ROESY Correlations (Dashed Arrow) for **2**

E-2, having *R* configuration at C-13.[7] Because protosappanin E-2 has been previously reported only as an inseparable mixture with E-1, their spectroscopic data has not been reported. Thus, this is the first report of the isolation and spectral data of protosappanin E-2 (**2**).

The HR-FAB-MS of compound **3** showed a quasimolecular ion at *m/z* 341.1001 $[M+Na]^+$, consistent with the molecular formula $C_{17}H_{18}O_6$. The IR spectrum of **3** displayed the absorbance of hydroxyl (3300 cm^{-1}) and phenyl (1600, 1445 cm^{-1}) groups. The ^{1}H-NMR spectrum of **3** revealed signals due to two methoxyl, an oxymethylene, an aliphatic methylene, two singlets of a 1,2,4,5-tetrasubstituted benzene ring, and three aromatic protons consisting of an ABX system (Table 2). In addition, its ^{13}C-NMR spectrum showed the signals of eight quaternary carbons consisting of four oxygenated sp^2 carbons (δ 158.5, 158.1, 144.6, 144.8), three aliphatic carbons (δ 127.2, 132.5, 121.0), and an acetal carbon (δ 100.5) (Table 2). These data were similar to those of protosappanin A (**7**),[10] isolated from the same extract, but they were characterized by the appearance of signals of two methoxyl groups and an acetal carbon and disappearance of the signal of a ketone carbonyl carbon in **7**. Thus, **3** was assumed to be protosappanin A dimethyl acetal, which was confirmed by the HMBC spectrum and the fact that an acid treatment of **3** gave **7**.

In this paper, we have identified a novel benzindenopyran, neoprotosappanin (**1**) and a novel dibenzoxocin, protosappanin A dimethyl acetal (**3**), and protosappanin E-2 (**2**) together with 14 known phenolic compounds (**4**—**17**). To the best of our knowledge, **1** represents a novel carbon framework, a benzindenopyran system, produced from two monomeric units, brazilin (**8**), while **3** might be an artifact formed from **7** during isolation procedure as demonstrated by the reverse reaction.

The isolated compounds were tested for their XO inhibitory activity (Table 3). The assay was carried out at five different concentrations ranging from 0.2—100 μM, and compounds **1**—**4**, **7**, **13**, and **15**—**17** displayed significant dose-dependent inhibition. Sappanchalcone (**17**) displayed the most potent activity with an IC_{50} value of 3.9 μM (Fig. 3), comparable to that of allopurinol (IC_{50}, 2.5 μM), a well known XO inhibitor clinically used for treatment of gout. Further literature survey revealed that this is the first report of chalcone on XO inhibitory activity. This suggests the need for further exploration of chalcones as the natural XO inhibitors for the possible health benefit in the treatment of

Table 3. Xanthine Oxidase Inhibitory Activity of the Isolated Compounds

Compounds	IC_{50} (μM)	Type of inhibition	K_i (μM)
1	38.3	Noncompetitive	29.2
2	18.9	Competitive	10.6
3	50.7	Competitive	26.9
4	29.7	Competitive	16.3
7	55.6	Competitive	34.7
13	93.2	Competitive	61.6
15	34.2	Competitive	20.7
16	36.8	Noncompetitive	27.4
17	3.90	Competitive	2.60
Allopurinol	2.50	Competitive	1.80

Other compounds showed only weak inhibitory activity at 100 μM, and thus are excluded in this Table.

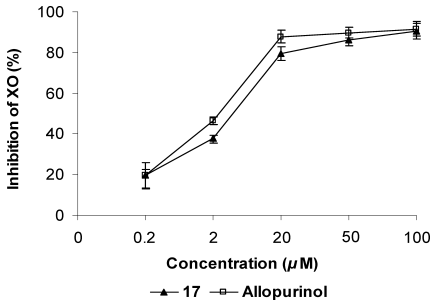

Fig. 3. Dose-Dependent Inhibition of XO by **17** and Allopurinol
The data represent the mean±S.D. of four different experiments.

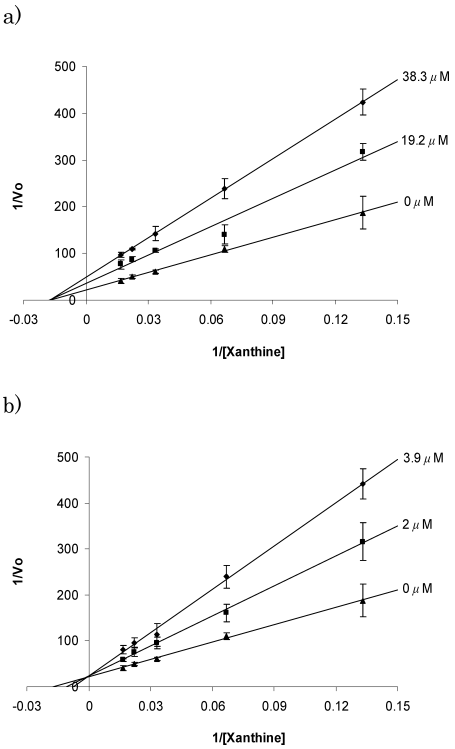

Fig. 4. Lineweaver–Burk Plots of the Inhibition of XO by (a) **1**, Noncompetitive Inhibitor, and (b) **17**, Competitive Inhibitor

gout. Among compounds possessing dibenz[*b*,*d*]oxocin carbon framework (**3**, **5**—**7**), those having dimethyl acetal (**3**) or carbonyl functionality (**7**) at C-3 were more active than others (**3**≈**7**≫**5**≈**6**). Brazilin-type monomers such as **8** and **9** were inactive, but the potency of XO inhibitory activity was significantly increased by the combination of two brazilin units or the brazilin unit with another dibenzoxocin (**2**>**1**>**3**≈**7**≫**5**≈**6**). However, the dimeric dibenzoxocin **4** with two similar units showed moderate inhibitory activity (**2**>**4**>**1**). The benzopyranol-type compounds **10**—**14** displayed only weak inhibitory activity, while benzopyranones showed significantly improved activity (**15**≈**16**≫**12**—**14**).

Further kinetic studies indicated **2**, **3**, **4**, **7**, **13**, **15**, and **17** to be competitive inhibitors, the same as allopurinol, while **1** and **16** were noncompetitive inhibitors with respect to the substrate, xanthine. The inhibition constants (K_i) were determined from the slopes of the Lineweaver–Burk plot for competitive inhibition, and intercept on vertical axis for noncompetitive inhibition (Fig. 4) and are listed in Table 3. Thus, the traditional use of *C. sappan* for the treatment of rheumatism and inflammatory diseases in Vietnam could be attributable to the XO inhibitory activity of phenolic constituents such as **17**.

Experimental

General Experimental Procedures Optical rotations were recorded on a JASCO DIP-140 digital polarimeter. IR spectra were measured with a Shimadzu IR-408 spectrophotometer in $CHCl_3$ solutions. NMR spectra were taken on a JEOL JNM-LA400 spectrometer with tetramethylsilane (TMS) as an internal standard, and chemical shifts are expressed in δ values. HR-FAB-MS measurements were carried out on a JEOL JMS-700T spectrometer, and glycerol was used as a matrix. Column chromatography was performed with BW-820MH Si gel (Fuji Silisia, Aichi, Japan). Analytical and preparative TLC were carried out on precoated Merck Kieselgel 60F_{254} or RP-18F_{254} plates (0.25 or 0.5 mm thickness).

Plant Material The heartwood of *C. sappan* was collected at the Seven-Mountain area, An Giang province, Vietnam, in October 2003 and was identified by Mr. Nguyen Duy Chinh, Department of Resource and Environment, Dalat University, Dalat City, Lam Dong province, Vietnam. A voucher sample (TMPW 23840) is preserved in the Museum for Materia Medica, Analytical Research Center for Ethnomedicines, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, Toyama, Japan.

Extraction and Isolation The dried heartwood of *C. sappan* was crushed into a powder and the powder (2.5 kg) was extracted with MeOH (12 l, reflux, 3 h, ×3) to yield a MeOH extract (300 g; IC_{50}, 16.9 μg/ml). The extract was partitioned between EtOAc and water to give an EtOAc-soluble fraction (170 g; IC_{50}, 12.8 μg/ml). The EtOAc-soluble fraction (100 g) was subjected to silica gel column chromatography with MeOH–$CHCl_3$ to give seven fractions: fr. 1, 5% MeOH–$CHCl_3$ eluate, 4.1 g; fr. 2, 7% MeOH–$CHCl_3$ eluate, 1.4 g; fr. 3, 8% MeOH–$CHCl_3$ eluate, 9.4 g; fr. 4, 9% MeOH–$CHCl_3$ eluate, 7.6 g; fr. 5, 12% MeOH–$CHCl_3$ eluate, 40 g; fr. 6, 15% MeOH–$CHCl_3$ eluate, 19.7 g; fr. 7, 20% MeOH–$CHCl_3$ eluate, 14 g. These fractions were examined for XO inhibitory activity, and active fractions were subjected to the further separation.

Fraction 2 (IC_{50}, 19.5 μg/ml) was rechromatographed on silica gel with MeOH–$CHCl_3$, followed by reversed-phase preparative TLC with CH_3CN : MeOH : H_2O=1 : 1 : 3, to give protosappanin A (**7**, 10.9 mg),[10] brazilin (**8**, 4.6 mg),[11] 3′-*O*-methylbrazilin (**9**, 2.9 mg),[12] 3′-deoxysappanol (**10**, 1.9 mg),[13,14] 3′-deoxy-4-*O*-methylsappanol (**11**, 2.5 mg),[15] and 4-*O*-methylsappanol (**12**, 11.6 mg).[12,16]

Fraction 3 (IC_{50}, 8.5 μg/ml) was further separated by silica gel comlumn chromatography, followed by reversed-phase preparative TLC with CH_3CN : MeOH : H_2O=1 : 1 : 3, to give neosappanone A (**4**, 3.3 mg), protosappanin A dimethyl acetal (**3**, 15.2 mg), **7** (21.6 mg), **8** (4.6 mg), **12** (23.7 mg), sappanol (**13**, 1.4 mg),[15] 4-*O*-methylepisappanol (**14**, 6.9 mg),[12,16] sappanone B (**15**, 8.0 mg),[12,15] 3-deoxysappanone B (**16**, 8.6 mg),[12,16] and sappanchalcone (**17**, 13.0 mg).[17]

Fraction 4 (IC_{50}, 11.9 μg/ml) was rechromatographed on silica gel with MeOH–$CHCl_3$, followed by reversed-phase preparative TLC with CH_3CN : MeOH : H_2O=1 : 1 : 3, to yield **8** (17.2 mg), **13** (3.9 mg), **14** (2.5 mg), and **16** (16.8 mg).

Fraction 5 (IC_{50}, 10.9 μg/ml) was subjected to silica gel comlumn chromatography with MeOH–$CHCl_3$, followed by reversed-phase preparative TLC with CH_3CN : MeOH : H_2O=1 : 1 : 3, to give protosappanin E-2 (**2**,

7.5 mg),[7] protosappanin B (**5**, 19.7 mg),[18] and protosappanin C dimethyl acetal (**6**, 8.6 mg).[9]

Fraction 6 (IC_{50}, 19.6 μg/ml) was separated by silica gel comlumn chromatography with MeOH–$CHCl_3$, followed by reversed-phase preparative TLC with $CH_3CN:MeOH:H_2O=1:1:3$, to yield neoprotosappanin (**1**, 2.8 mg) and **5** (12.2 mg).

Neoprotosappanin (**1**): Yellow amorphous solid; $[\alpha]_D^{25}$ −239.0° (c=0.30, MeOH). IR (KBr) cm^{-1}: 3300, 1610, 1450, 1350. ^{1}H- and ^{13}C-NMR (CD_3OD, 400 MHz), see Table 1. HR-FAB-MS m/z: 571.1586 $[M+H]^+$ (Calcd for $C_{32}H_{27}O_{10}$: 571.1604).

Protosappanin E-2 (**2**): Yellow amorphous solid; $[\alpha]_D^{20}$ −16.9° (c=0.175, MeOH). IR (KBr) cm^{-1}: 3300, 1640, 1600, 1450, 1380, 1270, 1180, 1070. ^{1}H- and ^{13}C-NMR (CD_3OD, 400 MHz), see Table 2. HR-FAB-MS m/z: 587.1558 $[M+H]^+$ (Calcd for $C_{32}H_{27}O_{11}$: 587.1553).

Protosappanin A Dimethyl Acetal (**3**): Light yellow amorphous solid; IR (KBr) cm^{-1}: 3300, 1600, 1445, 1350. ^{1}H- and ^{13}C-NMR (CD_3OD, 400 MHz), see Table 2. HR-FAB-MS m/z: 341.1001 $[M+Na]^+$ (Calcd for $C_{17}H_{18}O_6Na$: 341.0996).

Acid Treatment of 3 Compound **3** (3.0 mg) was treated with triflouroacetic acid (50 μl) in THF (2 ml) at room temperature for 3 h, followed by neutralization with sodium bicarbonate. The reaction mixture was extracted with ether and dried over anhydrous $MgSO_4$. The crude product was purified by preparative TLC with MeOH–$CHCl_3$ (1 : 9) to give **7**.

XO Inhibitory Assay The XO inhibitory activity was assayed spectrophotometrically at 290 nm under aerobic conditions by using 96-well plates as described previously.[6] XO inhibitory activity was expressed as the percentage inhibition of XO in the above assay system, calculated as $(1-B/A)\times100$, where A and B are the activities of the enzyme without and with test material. IC_{50} values were calculated from the mean values of data from four determinations.

Lineweaver–Burk Plots To determine the mode of inhibition by active compounds from the plant, Lineweaver–Burk plot analysis was performed. This kinetics study was carried out in the absence and presence of active compounds with varying concentrations of xanthine as substrate. The initial rates were determined on the basis of the rate of increase in absorbance at 290 nm between 0.5 and 3 min. The data represent the mean±S.D. of four determinations. The inhibition constants (K_i) were determined from the slopes of the Lineweaver–Burk plot for competitive inhibition, and intercept on vertical axis for noncompetitive inhibition.

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