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Neosappanone A, a xanthine oxidase (XO) inhibitory dimeric methanodibenzoxocinone with a new carbon skeleton from *Caesalpinia sappan*

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Abstract—A novel dimeric methanodibenzoxocinone, named neosappanone A (**1**), possessing a unique unprecedented novel carbon framework, has been isolated from the heartwood of *Caesalpinia sappan* L. of Vietnam, and its structure was elucidated on the basis of spectroscopic analysis. Neosappanone A (**1**) competitively inhibited xanthine oxidase in a concentration-dependent manner (IC_{50}, 29.7 μM; K_i, 16.3 μM).
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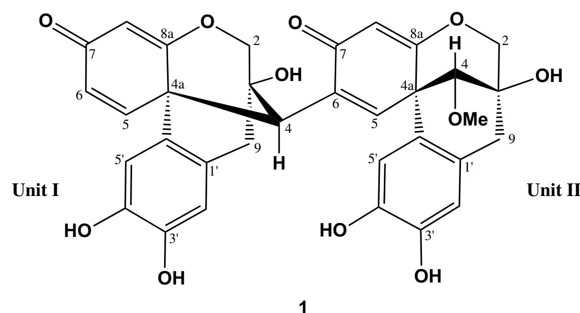
Gout is a common disease with a worldwide distribution. Hyperuricemia, which associates with gout, results from the overproduction or underexcretion of uric acid and is greatly influenced by a high dietary intake of foods rich in nucleic acids, such as meats (especially organ meats), leguminous seeds, some types of seafood, and food yeasts.[1,2] During the last step of purine metabolism, xanthine oxidase (XO) catalyzes the oxidation of xanthine and hypoxanthine into uric acid.[3] Uricosuric drugs, which increase the urinary excretion of uric acid or XO inhibitors, which block the terminal step in uric acid biosynthesis can lower the plasma uric acid concentration, and are generally employed for the treatment of gout.[4] Moreover, superoxide anion radicals generated by XO are involved in various pathological states such as hepatitis, inflammation, ischemia-reperfusion, carcinogenesis, and aging.[1,5] Therefore, the search for novel XO inhibitors would be beneficial not only to treat gout but also to combat various other diseases.

The dried heartwood of *Caesalpinia sappan* L. (Caesalpinaceae), called Sappan Lignum, is a well-known Vietnamese medicine, and has been used for the treatment of rheumatism and inflammatory diseases and as an emmenagogue, and homeostatic agent.[6] The heartwood of *C. sappan* has also been reported for its varied biological activities such as antioxidative, anti-inflammatory, hepatoprotective, cytotoxic, hypoglycemic, etc., which are attributed to the presence of phenolic compounds such as brazilin, chalcones, dibenz[*b,d*]oxocins, homoisoflavones, etc.[7,8] In the course of our screening program for XO inhibitory medicinal plants from Vietnam, we reported the inhibitory activity of 288 extracts, prepared from 96 medicinal plants used in Vietnamese traditional medicine to treat gout and related symptoms.[9] Among them, the methanolic extract of the heartwood of *C. sappan* exhibited significant XO inhibitory



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activity with an IC_{50} value of 14.2 μg/mL. Thus, further separation of the methanolic extract was carried out, which led to the isolation of a dimeric methanodibenzoxocinone named neosappanone A (**1**) with a novel carbon framework. In this paper, we report the structure elucidation of neosappanone A (**1**) together with its XO inhibitory activity.

Neosappanone A (**1**)[10] was obtained as a yellow amorphous solid and showed α_D^{25} +1.40 (*c* 0.15, MeOH). Its molecular formula was determined by HRFABMS to be $C_{33}H_{28}O_{11}$ [*m/z* 601.1709 $(M+H)^+$]. The IR spectrum of **1** showed the absorptions due to hydroxyl (3300 cm^{-1}), α,β-unsaturated carbonyl (1640 cm^{-1}), and phenyl (1600, 1450 cm^{-1}) groups. The ^1H NMR spectrum of **1** (Table 1) revealed signals due to a methoxyl, two oxygen-substituted methylenes, two aliphatic methylenes, an oxygen-substituted methine, an aliphatic methine, three isolated olefinic protons, and two vicinal olefinic protons, together with four singlets of two 1,2,4,5-tetrasubstituted benzene rings. Moreover, the ^{13}C NMR spectrum (Table 1) showed two ketone carbonyl carbons, eight olefinic carbons, and six oxygen-substituted carbons, together with 12 aromatic carbons. These ^1H and ^{13}C NMR signals appeared as related

Table 1. ^1H NMR and ^{13}C NMR data for (**1**) in CD_3OD (*J* values in parentheses)

Unit	Position	^1H	^{13}C
I	2	4.29 d (11.7); 4.22 d (11.7)	76.2
	3		68.5
	4	4.31 s	44.1
	4a		51.3
	5	6.85 d (10.0)	150.9
	6	6.46 dd (10.0, 1.7)	113.5
	7		191.2
	8	5.55 d (1.7)	109.0
	8a		179.2
	9	3.39 d (16.6); 3.17 d (16.6)	46.4
	1′		128.1
	2′	6.63 s	116.8
	3′		146.9
	4′		145.1
	5′	6.46 s	130.7
	6′		125.8
II	2	4.12 d (11.0); 3.77 d (11.0)	79.3
	3		71.0
	4	3.51 s	84.5
	4a		53.0
	5	7.10 s	144.9
	6		136.0
	7		189.2
	8	5.55 s	108.7
	8a		176.8
	9	3.29 d (16.1); 2.83 d (16.1)	38.8
	1′		128.0
	2′	6.53 s	116.2
	3′		146.2
	4′		144.5
	5′	6.10 s	114.6
	6′		123.7
	OMe	3.63 s	62.5

Figure 1. Key HMBC correlations (arrows) observed for **1**.

pairs, indicating that **1** has a dimeric carbon skeleton with two similar units (units I and II).

Unit I of neosappanone A (**1**) displayed the typical signals of an ABX system (δ_{H-5} 6.85, d, 10.0 Hz; δ_{H-6} 6.46, dd, 10.0, 1.7 Hz; δ_{H-8} 5.55, d, 1.7 Hz) and two aromatic singlets ($\delta_{H-2'}$ 6.63, $\delta_{H-5'}$ 6.46), together with two methylenes (δ_{H-2} 4.29, 4.22; δ_{H-9} 3.39, 3.17), and a methine (δ_{H-4} 4.31) proton in the ^1H NMR spectrum (Table 1). On the other hand, the ^{13}C NMR data for unit I indicated the presence of an α,β-unsaturated ketone carbonyl (δ_{C-7} 191.2), an oxygenated quaternary carbon (δ_{C-3} 68.5), an aliphatic quaternary carbon (δ_{C-4a} 51.3), an sp^2 quaternary carbon (δ_{C-8a} 179.2), and two oxygenated aromatic carbons ($\delta_{C-3'}$ 146.9, $\delta_{C-4'}$ 145.1) (Table 1). These data were similar to those of caesalpin J (**2**), previously reported as a constituent of the same plant species *C. sappan* from Japan,[11,12] except for the disappearance of signals due to a methoxyl group at C-4. This was further confirmed by HMBC spectral analysis (Fig. 1), which led to the construction of monomeric unit I.

Unit II, on the other hand, also displayed the signals similar to those of **2** in the ^1H and ^{13}C NMR spectra, except for the disappearance of signals due to an ABX system in **2** and appearance of two singlets (δ_{H-5} 7.10 and δ_{H-8} 5.55) in **1**. This suggested that the two singlets in unit II are *para* to each other and that the linkage to unit I is via C-6. Further HMBC analysis of unit II led to the complete assignment as shown in Figure 1, and the linkage between units I and II was determined to be between C-4 in unit I and C-6 in unit II by the HMBC correlations of H-4 in unit I with C-5, C-6, and C-7 in unit II (Fig. 1).

The relative stereochemistry of **1** was assigned on the basis of the ROESY correlations (Fig. 2). In unit I, significant ROESY correlations between H-4 and H-9 (δ_H 3.39), between H-4 and H-5, and between H-5 and H-5′ indicated the α-equatorial orientation of H-4. Similarly, ROESY correlations between H-9 (δ_H 3.17) and H-2 (δ_H 4.29), indicated the β-orientation of the 3-OH substituent (Fig. 2a). As for unit II, ROESY correlations between H-4 and H-2 (δ_H 3.77), between OCH_3 at C-4 and H-5, between H-5 and H-5′, and between H-9 (δ_H 2.83) and H-2 δ_H 4.12) indicated the β-axial orientation of H-2 and H-4, and β-orientation of the 3-OH substituent (Fig. 2b). Further analysis of the ROESY

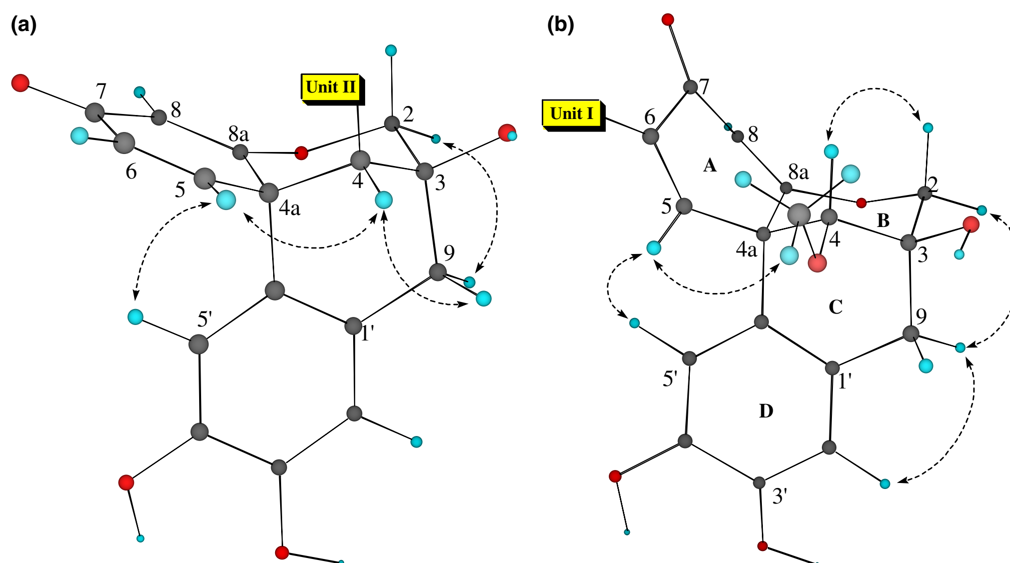
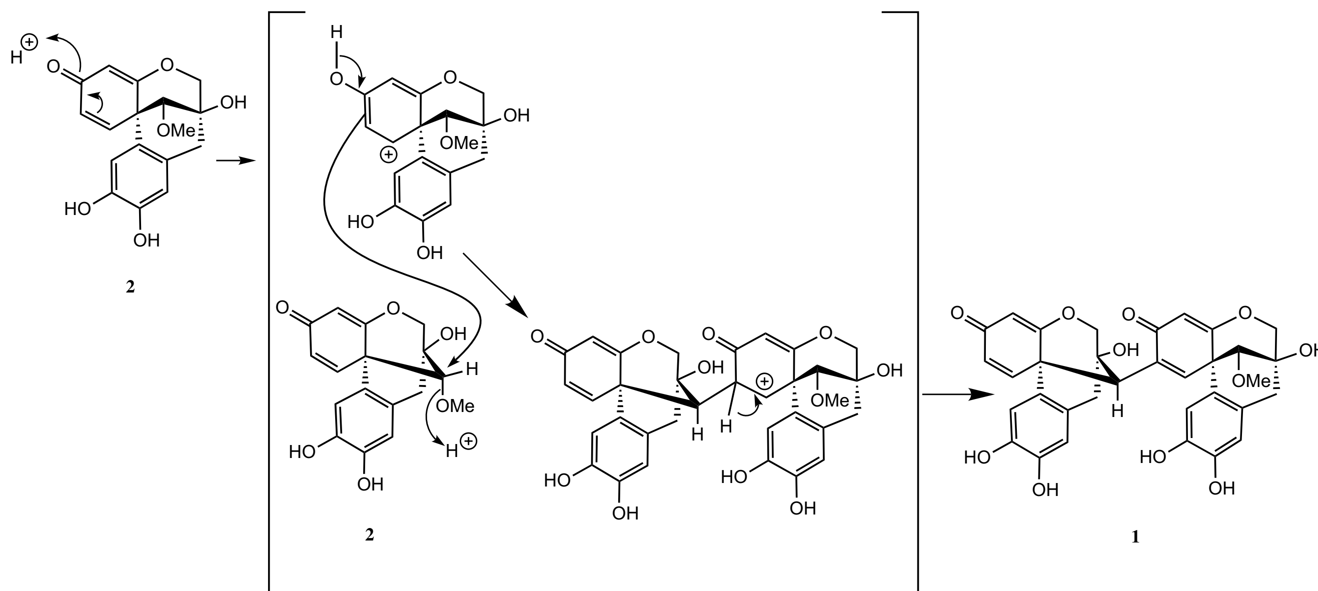


Figure 2. ROESY correlations observed in (a) unit II and (b) unit I.



Scheme 1. Possible biogenetic scheme of (**1**) from caesalpin J (**2**).

correlations by means of the Drieding model indicated the chair conformation of ring B. Thus, the stereostructure of neosappanone A was concluded as **1** (Fig. 2).

To the best of our knowledge, **1** represents a biogenetically unique novel carbon framework, a methanodibenzoxocinone system, which may be produced from two monomeric units, caesalpin J (**2**),[11] (Scheme 1) by S_N2 mechanism.

Neosappanone A (**1**) was tested for the XO inhibitory activity,[13] and displayed concentration-dependent inhibition with an IC_{50} value of 29.7 μM, while the positive control, allopurinol, revealed the IC_{50} value of 2.6 μM. Further kinetic studies indicated **1** to be a competitive inhibitor with respect to the substrate, xanthine, as allopurinol (Fig. 3). The inhibition constant (K_i) was

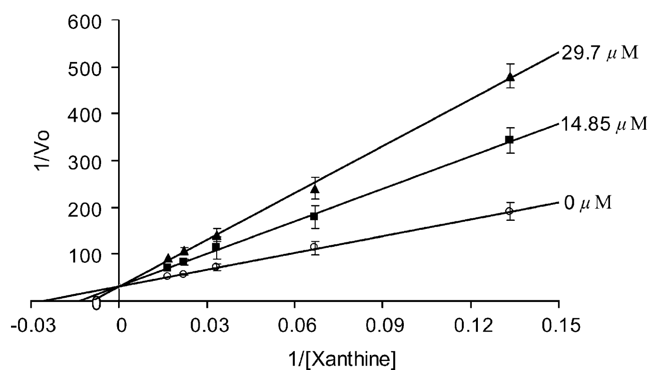


Figure 3. Lineweaver–Burk plots for XO inhibition of **1**. The initial rates were determined from the rate of increase in absorbance at 295 nm between 0.5 and 3 min. The data represent the mean ± SD of four determinations.

determined from the slopes of the Lineweaver–Burk plot against inhibitor concentration and found to be 16.3 μM for **1**, while that of allopurinol was 1.88 μM.

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10. Yellow amorphous solid, $[\alpha]_D^{25}$ +1.40 (*c* 0.15, MeOH). CD λ_{max} (1.67×10^{-4} M, EtOH) nm: 327 ([θ] +96,749), 282 ([θ] +83,827). IR ν_{max} (KBr) 3300, 1640, 1600, 1450, 1380, 1270, 1180, 1070 cm^{-1}. HRFABMS 601.1709 [calcd for $C_{33}H_{29}O_{11}(M+H)^+$, 601.1710]. ^1H and ^{13}C NMR, see Table 1.
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13. The XO inhibitory activity was assayed spectrophotometrically under aerobic conditions by using 96-well plates. The assay mixture consisting of 50 μL of test solution, 35 μL of 70 mM phosphate buffer (pH = 7.5), and 30 μL of enzyme solution (0.01 units/mL in 70 mM phosphate buffer, pH = 7.5) was prepared immediately before use. After preincubation at 25 °C for 15 min, the reaction was initiated by the addition of 60 μL of substrate solution (150 μM xanthine in the same buffer). The assay mixture was incubated at 25 °C for 30 min. The reaction was stopped by adding 25 μL of 1 N HCl, and the absorbance at 290 nm was measured with a Perkin–Elmer HTS-7000 Bio Assay Reader (Norwalk, CT, USA). A blank was prepared in the same way, but the enzyme solution was added to the assay mixture after adding 1 N HCl. One unit of XO is defined as the amount of enzyme required to produce 1 μmol of uric acid/min at 25 °C.