Koboquinone A and B, New Metabolites of Kobophenol A in Rats

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Two new phase I metabolites of phytoestrogen kobophenol A (1), called koboquinone A (2) and B (3), have been isolated from the feces of rats orally administered 1. Their structures were determined by spectroscopic methods. 2 also showed activity of stimulating the proliferation of cultured osteoblasts.

Key words metabolite; phytoestrogen; proliferation; osteoblast

Caragana sinica (Buc'hoz) Rehd. (Fabaceae) is widely distributed in China. Its dried roots (Chinese name: Jinquegen) have been used in China as a folk medicine for the treatment of asthenia syndrome, vascular hypertension, leukorrhagia, bruises and contused wounds. In our previous study, we found that the EtOAc extract of the roots contained many oligostilbenes which had multi-faceted bioactivities.[1] Using improved E-screen assay, we found that kobophenol A (**1**) is a phytoestrogen.[2]

Further studies on the pharmacokinetics of **1**, have yielded the metabolites koboquinone A (8a-(11a-hydroxyl-10a,13a-*p*-benzoquinonyl)-kobophenol A analog) (**2**) and koboquinone B (8d-(11d-hydroxyl-10d,13d-*p*-benzoquinonyl)-kobophenol A analog) (**3**) (Fig. 1), which are described for the first time.

Compound **2** was isolated as a reddish powder with a molecular formula of $C_{56}H_{42}O_{14}$ by high resolution electrospray ionization mass spectrometry (HR-ESI/MS). Its ^{1}H-NMR spectrum (Table 1), compared to that of **1**, also exhibited signals for the four 4-hydroxyl-1-substituted benzyl moieties, two 3,5-dihydroxy-1,2-disubstituted benzyl moieties and eight aliphatic protons. However, it only showed one 3,5-dihydroxyl-1-substituted benzyl moiety, while that of **1** has two 3,5-dihydroxy-1-substituted benzyl moieties. The other was changed into a 1,2,3,5-tetrasubstituted moiety. This new structure fragment might be a *para*-quinone structure oxidated from **1** deduced from the molecular weight fourteen units higher than that of **1**. This hypothesis was also supported by its ^{13}C-NMR spectrum with resonances at δ 188.0 and 188.1, assigned to a *para*-quinone system. Correlations of C-10a with H-12a and H-14a in the heteronuclear multiple bond correlation (HMBC) spectrum were in agreement with the presence of such a system.

Compound **3** also was isolated as a reddish powder with a molecular formula of $C_{56}H_{42}O_{14}$ by HR-ESI/MS. Though its ^{13}C-NMR spectral data (Table 1) was very similar to that observed for **2**, its ^{1}H-NMR spectrum was very different. Chemical shift values of proton signals of **3** differed from those of **2** about 0.1 to 0.2 ppm more or less randomly. The sequence of part aliphatic proton signals of **3** on the ^{1}H-NMR spectrum was H-8d, H-8b and H-8c from downfield to high field while that of **2** was H-8b, H-8c and H-8d. Compared to that of **1**, the ^{1}H-NMR spectrum also exhibited four 4-hydroxyl-1-substituted benzyl moieties and two 3,5-dihydroxy-1,2-disubstituted benzyl moieties. However, similar to that of **2**, it only showed one 3,5-dihydroxyl-1-substituted benzyl moiety, while the ^{1}H-NMR spectrum of **1** has two. The other was changed into a 1,2,3,5-tetrasubstituted moiety. Since the 1,2,3,5-tetrasubstituted moiety of **2** represents a *para*-quinone structure at ring A2, this moiety of **3** should stand for another *para*-quinone structure at ring D2 which had been a 3,5-dihydroxyl-1-substituted benzyl moiety. The MS/MS spectrum of **3** at *m/z* 937.21 gave strong peaks at *m/z* 815, 797, 708. The peak at *m/z* 815 represented the loss of the *para*-quinone structure (ring D2) from the M^{-}, and additionally loss of H_2O gave a peak at *m/z* 797. The peak at *m/z* 708 represented the loss of the 3,5-dihydroxyl-1-substituted benzyl moiety (ring A2) from the fragment of *m/z* 815. Moreover, **2** and **3** showed a difference in TLC and *Rf* values.

We consider metabolites **2** and **3** of interest since their structures are different from those naturally occurring stilbene tetramers which have a quinone structure or two or

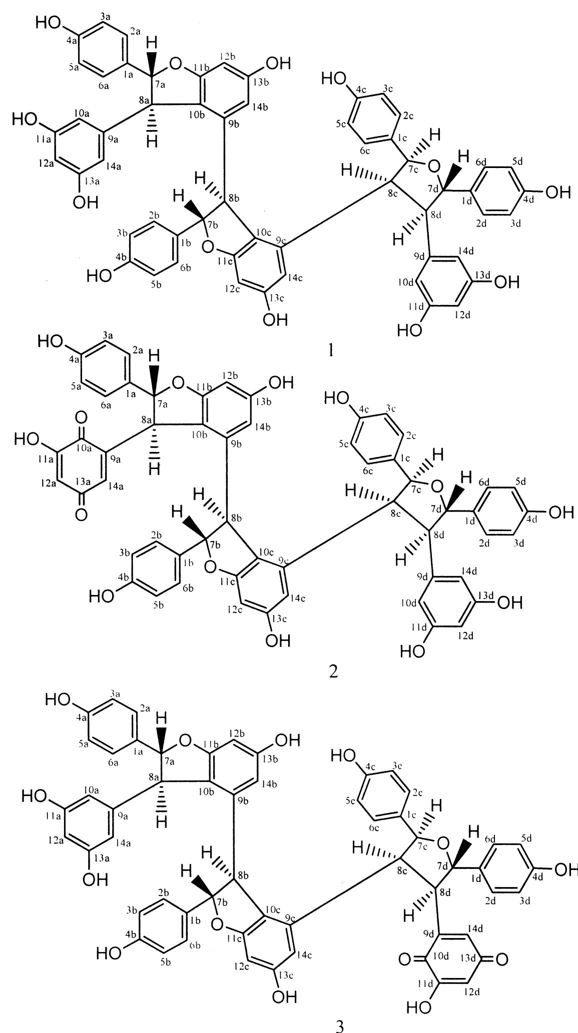

Fig. 1. Chemical Structures of Compounds **1**—**3**

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Table 1. NMR Spectral Data for **2**, **3** in CD_3OD

Position	**2**			**3**	
	^{1}H(δ)	^{13}C(δ)	HMBC	^{1}H(δ)	^{13}C(δ)
1a		134.5	3(5)a, 8a		134.4
2(6)a	7.57 d (8.4)	127.4	2(6)a, 7a	7.44 d (8.5)	126.3
3(5)a	6.78 d (8.6)	116.5	3(5)a	6.76 d (8.6)	116.9
4a		158.1	2(6)a		158.5
7a	5.40 s	89.9	2(6)a	5.52 s	92.9
8a	4.46 s	51.5	14a	4.44 s	58.4
9a		145.6	7a		148.4
10a		188.1	12(14)a	6.21 d (2.0)	107.4
11a		172.6			160.4
12a	5.32 d (2.6)	107.0		6.11 t (2.1)	98.9
13a		188.0			160.4
14a	5.50 d (1.7)	138.5	12a	6.21 d (2.0)	107.4
1b		133.7	3(5)b, 8b		134.1
2(6)b	6.42 d (8.9)	128.4	2(6)b, 7b	6.13 d (8.6)	127.5
3(5)b	6.46 d (8.6)	116.6	3(5)b	6.39 d (8.5)	116.6
4b		158.0	2(6)b		157.3
7b	5.21 d (4.3)	94.8	2(6)b	5.12 d (2.0)	93.0
8b	3.36 d (4.6)	54.0	14b	3.44 d (2.0)	51.5
9b		145.0	7b		144.3
10b		115.4	8b, 7a		120.2
11b		162.3	7a, 8a		162.4
12b	6.45 d (1.9)	97.2	14b	6.47 d (2.0)	97.0
13b		162.1			161.4
14b	6.01 d (2.2)	109.4	8b, 12b	5.94 d (2.0)	108.7
1c		131.7	3(5)c, 7c		131.1
2(6)c	6.34 d (8.4)	127.7	2(6)c	6.17 d (8.6)	128.0
3(5)c	6.53 d (8.6)	115.5	3(5)c	6.46 d (8.6)	115.6
4c		156.0	2(6)c		156.2
7c	4.77 d (4.1)	85.6	2(6)c	4.74 d (4.1)	86.1
8c	3.15 dd (6.0, 4.8)	53.1	2(6)c, 8d	3.15 dd (6.3, 4.6)	52.2
9c		136.5	7c, 8c, 8d		135.3
10c		123.9	8c, 8b, 12c, 14c		124.2
11c		160.7	8b		161.4
12c	5.95 d (1.9)	96.0	14c	6.07 d (2.0)	96.8
13c		158.9			159.3
14c	6.32 d (2.2)	111.1	8c, 12c	6.18 d (2.0)	110.7
1d		133.7	3(5)d, 8d		131.9
2(6)d	7.01 d (8.6)	128.9	2(6)d	7.10 d (8.5)	129.1
3(5)d	6.71 d (8.4)	116.3	3(5)d	6.76 d (8.6)	116.6
4d		158.3	2(6)d		158.2
7d	5.13 d (10.6)	86.2	2(6)d, 8c	4.39 d (10.9)	86.2
8d	3.01 dd (10.3, 6.0)	62.4	10(14)d	3.77 dd (11.2, 6.5)	54.4
9d		139.5	8d		140.0
10d	5.71 d (2.2)	109.1	8d, 10(14)d, 12d		190.7
11d		103.1			181.3
12d	5.97 t (2.2)	103.1	10(14)d	5.18 d (2.2)	102.4
13d		158.3			188.5
14d	5.71 d (2.2)	109.1	8d, 10(14)d, 12d	5.60 d (2.4)	136.1

more carbonyl groups.[3,4] Stilbene tetramer stenophyllol A,[3] an oxidative derivative of (−)-hopeaphenol, has the *para*-quinone structure at ring C1 which used to be a 4-hydroxyl-1-substituted benzyl moiety but not a 3,5-dihydroxyl-1-substituted benzyl moiety. Stilbene tetramer leachianol C has two carbonyl groups at ring B2 but they are seated at the *meta* position and cannot form a quinine structure.[4] Furthermore, **2** also has the activity to stimulate the proliferation of osteoblasts. Compound **3** was not tested due to insufficient sample size.

Experimental

General Experimental Procedures UV spectra were obtained in absolute MeOH on a Shimadzu UV-260 spectrophotometer. IR spectra were taken on an Avatar 360 E.S.P. Fourier Transform Infrared Spectroscope. The optical rotation was determined on a JASCO P-1020 polarimeter in MeOH. ^{1}H-, ^{13}C- and two dimension spectra were taken in CD_3OD on a Mercury Plus 400 NMR spectrometer. HR-ESI/MS/MS was recorded on a Q-Tof micro spectrometer. Sephadex LH-20 (Pharmacia) and Lobar RP-18 (40—63 μm, Merck) were used for column chromatography. ODS-2 hypersil C18 reversed-phase column (5 μm, 15×0.46 cm) was used for HPLC analysis.

Material Kobophenol A (**1**) (Fig. 1) was isolated from the roots of *Caragana sinica* accorded to the methods we reported,[1] 13.6 g (96.4%, measured by HPLC analysis); Structurally confirmed by comparing its ^{1}H- and ^{13}C-NMR data with those reported.[5]

Animals and Treatment Animal studies were conducted with approval of the Institutional Animal Care and Use Committee. Ten Sprague–Dawley rats (body weight 200—250 g) were fasted for 12 h before being dosed in stainless steel metabolic cages with free access to water. Each of them was administered 100 mg **1** (dissolved in 1 ml 40% ethanol) orally through a stomach gavage needle and underwent the same procedure once more after a 12 h lag time.

Extraction and Isolation One hundred and forty grams of dried feces samples were soaked with 700 ml methanol for 3 d, giving 8 g of dried extract. The extract was dissolved in 40 ml methanol and submitted to LH-20 chromatography, using methanol as eluant. This gave 180 fractions, which were grouped after thin layer chromatography. Fractions 135—180 (577 mg), after being subjected to one Lobar RP-18 column eluted with MeOH–H_2O (2 : 3), were isolated and identified. **2** (13 mg) and **3** (3 mg) were identified that showed reddish coloration in both solution and solid state. After elution with MeOH–H_2O (3 : 2), **1** (215 mg) was isolated and identified.

Koboquinone A (**2**): Red amorphous powder: mp >240 °C; $[\alpha]_D^{20}$ +289.0° (c=0.155, MeOH); UV (MeOH) λ_{max} (log ε) 278 (3.9); IR (KBr) ν_{max} 3441, 2926, 1615, 1515, 1455, 1248, 1169, 1121, 1003, 838 cm^{-1}; ^1H-, ^{13}C-NMR, HMBC experiments (see Table 1); Negative HR-ESI/MS m/z 938.2567 $[M]^-$ (Calcd for $C_{56}H_{42}O_{14}$, 938.2575).

Koboquinone B (**3**): Red amorphous powder: mp >240 °C; $[\alpha]_D^{20}$ +313.7° (c=0.095, MeOH); UV (MeOH) λ_{max} (log ε) 280 (3.9); IR (KBr) ν_{max} 3419, 2923, 1615, 1515, 1453, 1245, 1170, 1120, 1003, 822 cm^{-1}; ^1H-, ^{13}C-NMR experiments (see Table 1); Negative HR-ESI/MS m/z 937.2502 $[M-H]^-$ (Calcd for $C_{56}H_{41}O_{14}$, 937.2496).

Assay for Stimulating the Proliferation of Osteoblasts The method of MTT was used to observe the activity of stimulating the proliferation of osteoblasts.[6] It was found that **2**, together with **1**, had the effect of stimulating the proliferation of cultured osteoblasts (the reproduction rate of osteoblasts was raised 15.3% and 21.6%, respectively at 1.0 μg/ml) over that of the control group.

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