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TWO AROMATIC COMPOUNDS RELATED TO BRAZILIN FROM *CAESALPINIA SAPPAN*

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Key Word Index—*Caesalpinia sappan*; Leguminosae; heartwood; Sappan Lignum; brazilin; antihypercholesteremic activity.

Abstract—Two new aromatic compounds structurally related to brazilin were isolated and identified from the heartwood of *Caesalpinia sappan*. They possessed antihypercholesteremic activity.

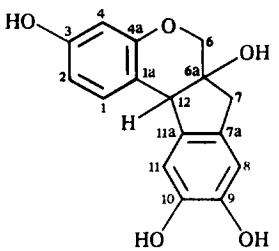
INTRODUCTION

Sappan Lignum, the dried heartwood of *Caesalpinia sappan* L. is known in oriental medicine, for example, as an anti-inflammatory agent. In the course of systematic screening for antihypercholesteremic activity, the methanolic extract of this plant was shown to exhibit predominant activity. This paper deals with the essential data leading to the assignment of structures **2** and **3** to the new compounds, together with already reported brazilin (**1**) [1], obtained from the methanolic extract of Sappan Lignum.

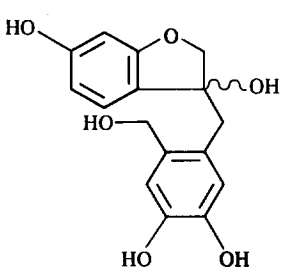
RESULTS AND DISCUSSION

The methanolic extract was further treated with cold methanol to separate a soluble portion from an insoluble portion, the former being chromatographed over Polyamide, Sephadex LH-20, Diaion and silica gel to afford compounds **1**–**3**.

Compound **1**, an amorphous powder, $[\alpha]_D$ +55.9° (MeOH), showed a $[M]^+$, $C_{16}H_{14}O_5$, at *m/z* 286 in the mass spectrum. The ^1H NMR spectrum of **1** exhibited the following signals due to ■–CH_2–■ (2H, AB*q*, J = 16 Hz) at δ2.74 and 3.03, ■–CH_2–O–■ (2H, AB*q*, J = 12 Hz) at



brazilin **1**



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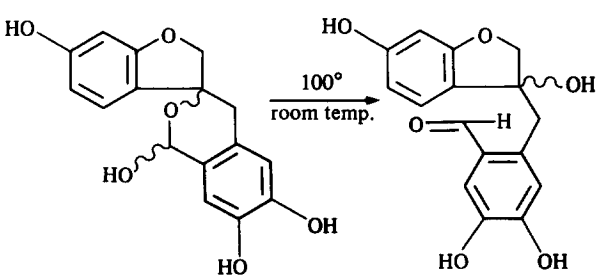

3

δ3.65 and 3.90, ■–CH–■ (1H, *s*) at δ3.96, five aromatic protons [(1H, *br s*) at δ6.26, (1H, *br d*, J = 8 Hz) at δ6.44, (1H, *s*) at δ6.57, (1H, *s*) at δ6.68 and (1H, *d*, J = 8 Hz) at δ7.16], which were reminiscent of brazilin and could be assigned to H_2-7, H_2-6, H-12, H-4, H-2, H-8, H-11 and H-1, respectively. The ^{13}C NMR spectrum of **1** could be well assigned without inconsistency as listed in Table 1. Compound **1** was, thus, deduced to be identical with brazilin.

Compound **2**, an amorphous powder, $[\alpha]_D$ −8.9° (MeOH), showed a $[M]^+$, $C_{16}H_{16}O_6$, at m/z 304 in the mass spectrum, corresponding to the one water-additional analogue of **1**. While measurement of the ^{13}C NMR spectrum in deuterated methanol at room temperature resulted in splitting or broadening of the signals, a satisfactory spectrum was recorded by measurement at 100° in DMSO-d_6. A comparative study of the ^{13}C NMR spectrum (Table 1) of **2** with that of **1** provided evidence concerning the structure of **2**. A significant difference between **1** and **2** in the ^{13}C NMR spectra was the appearance of a signal at δ66.0 (off-resonance: *t*) in the spectrum of **2** instead of a signal at δ51.0 (off-resonance: *d*) observed in that of **1**. Therefore, the above evidence indicates that the most probable structure for **2** is as shown. To verify this, **2** was first converted into the corresponding trimethyl ether, $[\alpha]_D$ −48.0° ($CHCl_3$), mass spectrum (*m/z*): 346 $[M]^+$, upon treatment with diazomethane. Its ^{1}H NMR spectrum (in deuterochloroform) showed a hydroxymethyl signal at δ3.54 (2H, *br s*) ascribable to H_2-12. The trimethyl ether was then acetylated with acetic anhydride–pyridine at room temperature to yield the monoacetate, $[\alpha]_D$ −69.5° ($CHCl_3$), whose mass spectrum exhibited a $[M]^+$ at m/z 388 indicating that one acetyl group was introduced into the primary alcohol. The monoacetate was further acetylated with acetic anhydride–*p*-TsOH to afford the diacetate, whose mass spectrum showed a $[M]^+$ at m/z 430, suggesting that **2** possessed one tertiary alcohol. Consequently, the structure for **2** was supported by the above reaction.

Compound **3**, an amorphous powder, $[\alpha]_D$ −34.0° (MeOH), showed a $[M]^+$, $C_{16}H_{14}O_6$, at m/z 302, corresponding to the dehydro analogue of **2**. The ^{1}H NMR spectrum also suggested that **3** was related to **2**. Though measurement of the ^{13}C NMR spectrum at room temperature in deuterated methanol brought about a little complexity in the signal pattern, it afforded sharp spectra (Table 1) at 55° in deuterated methanol or at 100° in DMSO-d_6. The respective chemical shifts of **3** measured at room temperature showed similar values compared with those of **2** except for that ascribable to C-12. The signal due to C-12 appeared at δ101.8 (off-resonance: *d*) in the measurement at room temperature and weakened slightly in intensity at 55°, whereas it disappeared at *ca* δ101 and a new signal occurred at δ202.8 (off-resonance: *d*) at 100°. In the measurement of the ^{1}H NMR spectrum at 100° in DMSO-d_6 a *s* was observed at δ9.75 suggesting the presence of an aldehyde proton. It was assumed that acetal ring opening would take place to afford the aldehyde compound at 100° in DMSO-d_6. Moreover, reduction of **3** with sodium borohydride in methanol gave a product identical with **2** in terms of TLC, IR and ^{13}C NMR spectra. Accordingly, the structure for **3** could be deduced as shown in the formula. The stereochemistry of C-6a in **2**, and C-6a and C-12 in **3** remains unsolved. Compounds **2** and **3** along with brazilin (**1**) are recognized

Table 1. ^{13}C NMR data for compounds **1**–**3** [δ (ppm)]

	1	**2**	**3**		
	in CD_3OD		in CD_3OD		
C No.	at room temp.	in DMSO-d_6 at 100°	at room temp.	at 55°	in DMSO-d_6 at 100°
1	132.6 (*d*)	131.5	132.8	132.8	132.0
1a	115.5 (*s*)	122.7	125.2	125.2	121.8
2	110.0 (*d*)	110.7	111.9	112.0	110.9
3	155.5 (*s*)	157.8	160.0	160.0	157.9
4	104.3 (*d*)	107.5	108.7	108.8	107.2
			108.8		
4a	157.5 (*s*)	158.4	159.1	159.1	157.6
6	70.8 (*t*)	75.5	78.1	77.5	72.9
			78.8		
6a	78.1 (*s*)	71.5	74.7	74.9	75.6
			74.8		
7	42.7 (*t*)	38.6	38.6	38.7	37.8
7a	131.4 (*s*)	130.4	132.5	132.7	130.7
8	112.9 (*d*)	119.0	120.2	120.2	119.1
				120.3	
9	145.4 (*s*)	143.6	145.0	144.9	144.2
10	145.1 (*s*)	143.7	145.0	144.8	143.9
11	112.5 (*d*)	116.7	117.6	117.8	116.9
11a	137.4 (*s*)	126.7	127.9	128.0	124.4
			128.0	128.1	
12	51.0 (*d*)	66.0 (*t*)	101.8 (*d*)	101.8 (*d*)	202.8 (*d*)
				204.5 (*d*)	

as characteristic substances possessing a rare new framework amongst naturally occurring compounds. Moreover, it has been found that compounds **2** and **3** are effective for hypercholesteremia. Details of the pharmacological tests will be reported elsewhere.

EXPERIMENTAL

^{1}H NMR (100 MHz) and ^{13}C NMR (50.3 MHz): TMS as int. standard; CC: Si gel (Merck 70–230, 230–400 mesh); TLC: precoated silica gel plates F_{254} (Merck). Spots were visualized by UV (254 nm) and 10% H_2SO_4 (Δ).

Extraction and isolation. The MeOH extract (50 g) was treated with MeOH (100 ml) at room temp. and separated into insoluble and soluble portions, the former being subjected to CC on Si gel ($CHCl_3$–MeOH, 20:1) to give fractions 1–6. Repeated CC of fractions 2–5 on silica gel ($CHCl_3$–MeOH, 20:1, 30:1), Sephadex LH-20 (MeOH), Polyamide (MeOH), Diaion (MeOH–H_2O, 1:1, 3:7) gave **1** (6.52 g), **2** (886 mg) and **3** (2.77 g).

Compound **1** (*brazilin*). R_f 0.32 ($CHCl_3$–MeOH–HOAc, 7:1:0.5), an amorphous powder. (Found: C, 67.31; H, 4.91. $C_{16}H_{14}O_5$ requires: C, 67.12; H, 4.93%.) $[\alpha]_D^{22}$ +55.9° (MeOH; *c* 1.02), IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1610, 1500 (aromatic ring), MS *m/z*: 286 $[M]^+$ ($C_{16}H_{14}O_5$), 268 $[M-H_2O]^+$, 229.

Tetraacetate of **1**. A mixture of **1** (150 mg), Ac_2O (5 ml) and *p*-TsOH (50 mg) was left to stand for 1 day at room temp. to afford the tetraacetate (137 mg). R_f 0.38 ($CHCl_3$), colourless needles. (Found: C, 63.16; H, 4.71. $C_{24}H_{22}O_9$ requires: C, 63.43; H, 4.88.) Mp 153–154° (from dilute MeOH), $[\alpha]_D^{21}$ +64.8° ($CHCl_3$; *c* 1.08), MS *m/z*: 394 $[M-HOAc]^+$, 352, 310, 268, ^{1}H NMR (in $CDCl_3$): δ2.06 (3H, *s*, OAc), 2.24 (6H, *s*, 2 × OAc), 2.26 (3H, *s*, OAc), 3.42 (2H, *s*, H_2-7), 3.70 (1H, *d*, *J* = 12 Hz, H-6β), 4.44 (1H, *br s*, H-12), 4.71 (1H, *dd*, *J* = 2, 12 Hz, H-6α), 6.64 (1H, *d*, *J* = 2 Hz, H-4), 6.78 (1H, *dd*, *J* = 2, 8 Hz, H-2), 7.03, 7.08 (each 1H, *s*, H-8, H-11), 7.35 (1H, *d*, *J* = 8 Hz, H-1).

Compound **2**. R_f 0.18 ($CHCl_3$–MeOH–HOAc, 7:1:0.5), an amorphous powder. (Found: C, 62.98; H, 5.28. $C_{16}H_{16}O_6$ requires: C, 63.15; H, 5.30%.) $[\alpha]_D^{22}$ −8.9° (MeOH; *c* 1.01), IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1605, 1500 (aromatic ring), MS *m/z*: 304 $[M]^+$ ($C_{16}H_{16}O_6$), 243, 229, 213, ^{1}H NMR (in DMSO-d_6 at 100°): δ3.31, 3.38 (each 1H, AB*q*, *J* = 11 Hz, H_2-12), 3.75, 3.98 (each 1H, AB*q*, *J* = 12 Hz, H_2-6), 6.41 (1H, *d*, *J* = 2 Hz, H-4), 6.51 (1H, *dd*, *J* = 2, 8 Hz, H-2), 6.61, 6.67 (each 1H, *s*, H-8, H-11), 6.91 (1H, *d*, *J* = 8 Hz, H-1).

Trimethyl ether of **2**. To a soln of **2** (392 mg) in EtOH (4 ml), CH_2N_2–Et_2O soln (15 ml) was added and left standing overnight at 5° giving the trimethyl ether (270 mg), R_f 0.10 (*n*-hexane–Me_2CO, 2:1), an amorphous powder. (Found: C, 65.78; H, 6.52. $C_{19}H_{22}O_6$ requires: C, 65.88; H, 6.40%.) $[\alpha]_D^{22}$ −48.0° ($CHCl_3$; *c* 1.00), MS *m/z*: 346 $[M]^+$ ($C_{19}H_{22}O_6$), 272 $[C_{16}H_{16}O_4]^+$, 257 $[C_{15}H_{13}O_4]^+$, ^{1}H NMR (in $CDCl_3$): δ2.58, 2.75 (each 1H, AB*q*, *J* = 13 Hz, H_2-7), 3.54 (2H, *br s*, H_2-12), 3.79, 3.86, 3.88 (each 3H, *s*, 3 × OMe), 4.31 (1H, *d*, *J* = 13 Hz, H-6), 6.61 (1H, *d*, *J* = 2 Hz, H-4), 6.69 (1H, *dd*, *J* = 2, 9 Hz, H-2), 6.77, 6.83 (each 1H, *s*, H-8, H-11), 7.18 (1H, *d*, *J* = 9 Hz, H-1). ^{13}C NMR (in $CDCl_3$): δ131.6, 124.7, 106.5, 158.5, 110.0, 160.5, 78.2, 71.9, 39.0, 131.6, 115.1, 148.1, 147.9, 113.3, 126.3, 67.2 (C-1, C-1a, C-2, C-3, C-4, C-4a, C-6, C-6a, C-7, C-7a, C-8, C-9, C-10, C-11, C-11a, C-12), 55.4, 56.0, 56.1 (3 × OMe).

Trimethyl monoacetate of **2**. Trimethyl ether (30 mg) was acetylated with pyridine (2 ml) and Ac_2O (2 ml) overnight at room temp. to afford the trimethyl monoacetate of **2** (23 mg), R_f 0.22 (*n*-hexane–Me_2CO, 2:1), an amorphous powder. (Found: C, 64.81; H, 6.21. $C_{21}H_{24}O_7$ requires: C, 64.94; H, 6.23%.) $[\alpha]_D^{21}$ −69.5° ($CHCl_3$; *c* 0.95), MS *m/z* 388 $[M]^+$, 328 $[M-HOAc]^+$, ^{1}H NMR (in $CDCl_3$): δ2.11 (3H, *s*, OAc), 2.69 (2H, *br s*, H_2-7), 3.80, 3.87, 3.89 (each 3H, *s*, 3 × OMe), 4.14 (2H, *s*, H_2-12), 4.30 (1H, *d*, *J* = 12 Hz, H-6), 6.60 (1H, *d*, *J* = 2 Hz, H-4), 6.68 (1H, *dd*, *J* = 2, 8 Hz, H-2), 6.77, 6.84 (each 1H, *s*, H-8, H-11), 7.18 (1H, *d*, *J* = 8 Hz, H-1).

Trimethyl diacetate of **2**. A mixture of trimethyl monoacetate (5 mg), Ac_2O (0.5 ml) and *p*-TsOH (1 mg) was reacted for 2 hr at room temp. to give the trimethyl diacetate of **2**. R_f 0.36 (*n*-hexane–Me_2CO, 2:1), an amorphous powder (3 mg), MS *m/z*: 430 $[M]^+$, 388 $[M-C_2H_2O]^+$, 370 $[M-HOAc]^+$, 346, 328, 295, 279, 271, 257.

Pentaacetate of **2**. A mixture of **2** (200 mg), Ac_2O (5 ml) and *p*-TsOH (60 mg) was allowed to stand for 1.5 hr at room temp. to yield the pentaacetate of **2** (83 mg), an amorphous powder. (Found: C, 60.48; H, 5.02. $C_{26}H_{26}O_{11}$ requires: C, 60.70; H, 5.09%.) $[\alpha]_D^{21}$ −76.8° ($CHCl_3$; *c* 1.12), MS *m/z*: 514 $[M]^+$, 472 $[M-C_2H_2O]^+$, 454 $[M-HOAc]^+$, 430, 412, 388, 370, 346, 328, 286, 268, 229, ^{1}H NMR (in $CDCl_3$): δ2.02, 2.08 (each 3H, *s*, 2 × aliphatic OAc), 2.19 (9H, *s*, 3 × phenolic OAc), 2.69, 3.47 (each 1H, AB*q*, *J* = 14 Hz, H_2-7), 4.38, 4.50 (each 1H, AB*q*, *J* = 9 Hz, H_2-12), 3.95, 4.81 (each 1H, AB*q*, *J* = 12 Hz, H_2-6), 6.92 (1H, *br s*, H-4), 6.95 (1H, *dd*, *J* = 2, 8 Hz, H-2), 7.05, 7.16 (each 1H, *s*, H-11), 7.20 (1H, *d*, *J* = 8 Hz, H-1).

Compound **3**. R_f 0.22 ($CHCl_3$–MeOH–HOAc, 7:1:0.5), an amorphous powder. (Found: C, 63.42; H, 4.51. $C_{16}H_{14}O_6$ requires: C, 63.57; H, 4.67%.) $[\alpha]_D^{22}$ −34.0° (MeOH; *c* 1.85), IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1605, 1495 (aromatic ring), MS *m/z*: 302 $[M]^+$ ($C_{16}H_{14}O_6$), 286, 272, 259, 244, 229, 213, 185. ^{1}H NMR (in CD_3OD): δ3.99, 4.43 (each 1H, AB*q*, *J* = 14 Hz, H-6α, H-6β), 6.50 (1H, *d*, *J* = 2 Hz, H-4), 6.56 (1H, *dd*, *J* = 2, 8 Hz, H-2), 6.70 (2H, *s*, H-11), 7.00 (1H, *d*, *J* = 8 Hz, H-1); (in DMSO-d_6, at 100°): δ2.57, 2.77 (each 1H, AB*q*, *J* = 13 Hz, H_2-7), 3.91, 4.17 (each 1H, AB*q*, *J* = 13 Hz, H-6α, H-6β), 6.44 (1H, *d*, *J* = 2 Hz, H-4), 6.55 (1H, *dd*, *J* = 2, 8 Hz, H-2), 6.65, 6.70 (each 1H, *s*, H-8, H-11), 6.96 (1H, *d*, *J* = 8 Hz, H-1), 9.75 (1H, *s*, H-12).

Reduction of **3** *with* $NaBH_4$. To a soln of **3** (71 mg) in MeOH (0.5 ml), $NaBH_4$ (10 mg) added and the reaction mixture left to stand for 1 hr at room temp. to give a product (48 mg), an amorphous powder, $[\alpha]_D^{21}$ −12.0° (MeOH; *c* 1.00), identical with **2** from its IR and ^{13}C NMR spectra.

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REFERENCE

1. Craig, J. C., Naik, A. R., Pratt, R. and Johnson, E. (1963) *J. Org. Chem.* **30**, 1573.