Coumestans from *Hedysarum multijugum*

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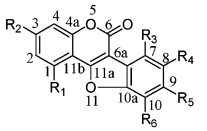
Ten coumestans were isolated from the roots of *Hedysarum multijugum*. Their structures were elucidated as the new hedysarimcoumestans A−H (**1**−**8**), 1,3,9-trimethoxycoumestan (**9**), and aureol (**10**), on the basis of spectroscopic analyses.

Hedysarum multijugum is a plant in *Hedysarum* Linn. of the family Leguminosae, which has been used as a folk herbal drug in northwest China and recorded in many Chinese herbal books. The roots of *H. multijugum* are used for the treatment of palpitation and chronic nephritis. Alzheimer's disease (AD) is a chronic, slowly progressive neurodegenerative disorder. A wide range of evidence has shown that acetylcholinesterase (AChE) inhibitors could retard progression of AD and improve cognition, behavior, and global function in AD.[1] In the course of searching for natural products to treat AD, we found that a 95% EtOH extract from the roots of *H. multijugum* improved the learning ability and memory of mice in vivo and showed remarkable acetylcholinesterase (AChE) inhibitory activity in vitro. Our previous phytochemical studies on the plant resulted in the isolation of some isoflavones and pterocarpenes.[2−4] Further studies led to the isolation of eight new coumestans, hedysarimcoumestans A−H (**1**−**8**), along with a new natural product, 1,3,9-trimethoxycoumestan (**9**), and a known coumestan, aureol (**10**), by repeated chromatography. The structures were identified on the basis of spectroscopic analyses.

Results and Discussion

The EtOAc- and *n*-BuOH-soluble parts of the 95% EtOH extract of *H. multijugum* were subjected to silica gel and Sephadex LH-20 column chromatography to afford compounds **1**−**10**.

Hedysarimcoumestan A (**1**) was obtained as purple needles. The molecular formula of **1** was determined by negative HRSI-MS as $C_{17}H_{12}O_6$. The IR spectrum showed bands at 3312, 1697, 1625, 1555, 1498, and 1330 cm^{-1} for the presence of hydroxyl, lactone carbonyl, and phenyl groups. UV data (λ_{max} 331, 262, 224 nm) indicated the coumestan character of **1**.[5] The ^{1}H NMR spectrum displayed five aromatic protons. Two *meta*-coupled protons belonging to a 1,2,3,5-tetrasubstituted aromatic ring at δ 6.45 (1H, d, J = 2.4 Hz) and 6.62 (1H, d, J = 2.4 Hz) were observed. Three protons at δ 7.75 (1H, d, J = 8.4 Hz), 7.05 (1H, dd, J = 8.4, 2.1 Hz), and 7.47 (1H, d, J = 2.1 Hz) revealed a typical ABX system. The signals at δ 3.84 (3H, s) and 3.81 (3H, s) were assigned to two *O*-methyl groups, and a signal at δ 11.07 (1H, s) was due to a hydroxyl group. The resonances at δ 6.45 and 6.62 were attributed to H-2 and H-4 of the A-ring, which generally resonate at a higher field. The signals at δ 7.75, 7.05, and 7.47 were attributed to H-7, H-8, and H-10 of the B-ring, which generally resonate at a lower field. Further, its ^{13}C NMR spectrum showed 17 carbon signals, including two *O*-methyl groups at δ 55.8 and 55.7. The IR, UV, ^{1}H NMR, and ^{13}C NMR spectra supported that **1** had a coumestan structure. In the HMBC spectrum of **1**, the *O*-methyl protons at δ 3.81 correlated with C-3 (δ 162.6), the aromatic protons at δ 6.45 (H-2) and 6.62 (H-4) correlated with C-3 (δ 162.6), and the hydroxyl proton at δ 11.07 correlated with C-11b (δ 96.3), indicating that the *O*-methyl group and the hydroxyl group were attached to C-3 and C-1 of the A-ring, respectively. The HMBC spectrum of **1** showed that the other *O*-methyl protons at δ 3.84 correlated with C-9 (δ 158.5), and the aromatic proton at δ 7.75 (H-7) correlated with C-9 (δ 158.5) and C-6a (δ 101.2), indicating that the *O*-methyl group was attached to C-9 (Figure 2). On the basis of detailed analysis of ^{1}H NMR, ^{13}C NMR, HSQC, and HMBC spectra, all the signals in the ^{1}H NMR and ^{13}C NMR spectra were assigned (Tables 1 and 2). The structure of **1** was elucidated as 1-hydroxy-3, 9-dimethoxycoumestan, named hedysarimcoumestan A.



1 R_1=OH, R_2=R_5=OMe, R_3=R_4=R_6=H
2 R_1=R_2=OH, R_5=OMe, R_3=R_4=R_6=H
3 R_2=R_5=OH, R_1=Oglc, R_3=R_4=R_6=H
4 R_1=R_2=OH, R_5=OMe, R_3=R_4=H, R_6=CH_2CH=$C(CH_3)_2$
5 R_1=R_2=OH, R_4=R_5=OMe, R_3=R_6=H
6 R_1=R_3=OH, R_2=R_5=OMe, R_4=R_6=H
7 R_1=R_2=R_3=OH, R_5=OMe, R_4=H, R_6=CH_2CH=$C(CH_3)_2$
8 R_1=R_2=R_3=OH, R_5=OMe, R_6=H, R_4=CH_2CH=$C(CH_3)_2$
9 R_1=R_2=R_5=OMe, R_3=R_4=R_6=H
10 R_1=R_2=R_5=OH, R_3=R_4=R_6=H

Figure 1. Structures of compounds **1**−**10**.

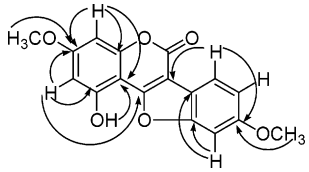


Figure 2. HMBC of **1**.

Hedysarimcoumestan B (**2**) was obtained as pale yellow needles with molecular formula $C_{16}H_{10}O_6$, which was established by HRSI-MS at *m*/*z* 297.0404 $[M - H]^-$ (calcd 297.0405). The IR and UV spectroscopic data indicated that the structure of **2** was similar to that of **1**. The ^{1}H NMR spectrum displayed five aromatic protons of the A- and B-rings at δ 6.39 (1H, d, J = 1.8 Hz), 6.42 (1H, d, J = 1.8 Hz), 7.76 (1H, d, J = 8.4 Hz), 7.08 (1H, dd, J = 8.4, 2.1 Hz), and 7.50 (1H, d, J = 2.1 Hz). The signal at δ 3.86 (3H, s) was assigned to an *O*-methyl group, and signals at δ 10.97 (1H, s) and 10.53 (1H, s) were due to two hydroxyl groups. In the ^{1}H and ^{13}C NMR spectra of **2**, the signals were similar to those of **1**. However, on comparison of the ^{1}H NMR spectra of **2** and **1**, the signal of the *O*-methyl group at δ 3.81 in **1** was missing in the spectrum of **2**, while one hydroxyl group at δ 10.53 was evident in **2**. In the HMBC spectrum, the correlation between *O*-methyl

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Table 1. ^{13}C NMR Data of Compounds **1**−**10** (in DMSO-d_6)

no.	1	2	3	4	5	6	7[a]	8[a]	9	10
1	155.0	155.4	154.3	156.0	155.7	155.0	155.2	155.3	156.8	155.2
2	98.1	99.2	98.8	99.5	99.5	98.5	99.7	100.0	96.2	99.1
3	162.6	161.6	161.4	162.1	161.8	162.6	161.8	162.2	163.5	161.4
4	93.2	95.1	96.8	95.3	95.3	93.5	95.5	95.8	94.5	94.9
4a	155.5	155.7	155.0	156.2	155.9	155.4	156.0	156.0	155.9	155.6
6	157.4	157.7	157.5	158.1	158.3	158.9	161.7	159.5	157.8	157.7
6a	101.2	100.6	101.6	101.0	101.4	101.9	101.8	102.0	102.3	100.7
6b	115.5	115.7	114.2	116.4	114.9	104.3	103.7	104.4	115.8	114.4
7	120.1	120.1	120.2	117.6	101.6	150.5	148.6	148.0	120.7	120.2
8	113.3	113.3	113.9	109.6	148.0	98.5	95.4	112.7	114.1	113.8
9	158.5	158.4	156.7	155.7	148.8	160.5	157.7	158.8	159.2	156.6
10	97.1	97.2	98.8	113.8	97.2	89.3	105.6	87.4	97.5	98.6
10a	155.8	155.8	156.0	154.2	149.6	156.8	155.5	155.2	156.3	155.8
11a	159.8	160.4	159.0	161.0	160.3	160.3	159.8	161.7	159.7	159.8
11b	96.3	95.0	96.3	95.6	95.7	96.5	95.5	96.2	97.3	95.2
1-OCH_3									57.1	
3-OCH_3	55.8					55.8			56.6	
8-OCH_3					56.6					
9-OCH_3	55.7	55.9		56.9	56.4	55.8	56.0	55.9	56.3	
glc-1′			100.2							isoprenyl
2′			73.1	22.9			22.0	22.1		1′
3′			76.7	121.6			122.1	123.1		2′
4′			69.4	132.3			131.2	130.6		3′
5′			77.2	18.1			17.0	17.4		4′
6′			60.5	26.0			25.2	25.5		5′

[a] Solvent: CD_3COCD_3.

Table 2. ^{1}H NMR Data of Compounds **1**, **2**, **3**, **9**, and **10** (in DMSO-d_6)

	δ [mult. *J* (Hz)]				
no.	1	2	3	9	10
2	6.45 (d, 2.4)	6.39 (d, 1.8)	6.65 (d, 2.1)	6.64 (d, 1.5)	6.36 (d, 1.8)
4	6.62 (d, 2.4)	6.42 (d, 1.8)	6.57 (d, 2.1)	6.77 (d, 1.5)	6.39 (d, 1.8)
7	7.75 (d, 8.4)	7.76 (d, 8.4)	7.69 (d, 8.1)	7.76 (d, 8.5)	7.65 (d, 8.4)
8	7.05 (dd, 8.4, 2.1)	7.08 (dd, 8.4,2.1)	6.94 (dd, 8.1, 1.5)	7.07 (dd, 2.0, 8.5)	6.91 (dd, 8.4, 2.1)
10	7.47 (d, 2.1)	7.50 (d, 2.1)	7.16 (d, 1.5)	7.46 (d, 2.0)	7.12 (d, 2.1)
1-OCH_3				4.00 (s)	
3-OCH_3	3.81 (s)			3.88 (s)	
9-OCH_3	3.84 (s)	3.86 (s)		3.85 (s)	
OH	11.07 (s)	10.97 (s)	10.72 (s)		10.90 (s)
		10.53 (s)	9.97 (s)		10.48 (s)
					9.94 (s)
glc-1′			5.12 (d, 7.5)		

protons at δ 3.86 and C-9 at δ 158.5 indicated linkage of the *O*-methyl group to C-9. The structure of **2** was thus elucidated as 1,3-dihydroxy-9-methoxycoumestan, named hedysarimcoumestan B.

Hedysarimcoumestan C (**3**) was obtained as pale yellow needles, and its molecular formula $C_{21}H_{18}O_{11}$ was established by HRSI-MS at *m*/*z* 445.0770 $[M - H]^-$ (calcd 445.0776) and 283.0249 $[M - glc]^-$. The IR and UV spectroscopic data indicated that the structure of **3** was similar to that of **1**. The ^{1}H NMR spectrum displayed five aromatic protons at δ 6.65(1H, d, *J* = 2.1 Hz), 6.57 (1H, d, *J* = 2.1 Hz), 7.69 (1H, d, *J* = 8.1 Hz), 6.94 (1H, dd, *J* = 8.1, 1.5 Hz), and 7.16 (1H, d, *J* = 1.5 Hz). The two hydroxyl protons resonated at δ 10.72 (1H, s) and 9.97 (1H, s). The signal at δ 5.12 (1H, d, *J* = 7.5 Hz) was due to the anomeric proton of the β-D-glucopyranose moiety. The ^{13}C NMR spectrum showed 21 carbon signals. In the HMBC spectrum (Figure 3), the aromatic proton at δ 6.65 (H-2) correlated with carbons at δ 161.4 (C-3), 154.3 (C-1), 96.3 (C-11b), and 96.8 (C-4), the aromatic proton at δ 6.57 (H-4) correlated with carbons at δ 161.4 (C-3), 155.0 (C-4a), 96.3 (C-11b), 98.8 (C-2), and 159.0 (C-11a), and the anomeric proton at δ 5.12 (H-1′) correlated with C-1 at δ 154.3, indicating that glucose should be attached at the C-1 position. One- and two-dimensional NMR techniques permitted the assignments of all proton and carbon signals of **3** (Tables 1 and 2). The structure of **3** was elucidated as 1,3,9-trihydroxycoumestan-1-*O*-β-D-glucopyranoside, named hedysarimcoumestan C.

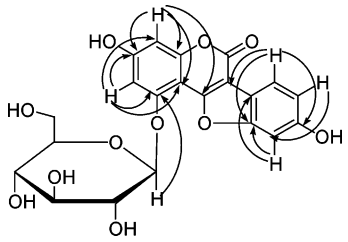

Figure 3. HMBC of **3**.

Hedysarimcoumestan D (**4**) was obtained as pale yellow needles, and its molecular formula ($C_{21}H_{18}O_6$) was established by HRSI-MS at *m*/*z* 365.1029 $[M - H]^-$ (calcd 365.1031). The IR and UV spectroscopic data indicated that the structure of **4** was similar to that of **1**. The ^{1}H NMR spectrum displayed four aromatic protons at δ 6.37(1H, d, *J* = 1.8 Hz), 6.36 (1H, d, *J* = 1.8 Hz), 7.62 (1H, d, *J* = 8.4), and 7.11 (1H, d, *J* = 8.4 Hz). The signal at δ 3.86 (3H, s) was assigned to an *O*-methyl group, and the protons at δ 10.90 (1H, s) and 10.48 (1H, a) were due to two hydroxyl groups. The protons at δ 1.83 (3H, s), 1.63 (3H, s), 3.56 (2H, d, *J* = 7.0 Hz), and 5.29 (1H, t, *J* = 7.0 Hz) indicated the presence of an isoprenyl group. The ^{13}C NMR spectrum showed 21 carbon signals. In the HMBC spectrum of **4**, the hydroxyl proton at δ 10.90 correlated with carbons at δ 156.0 (C-1), 99.5 (C-2), and 95.6 (C-11b) and the other hydroxyl proton at δ 10.48 correlated with carbons at δ 162.1 (C-3), 99.5 (C-2), and 95.3 (C-4), indicating

Table 3. ^{1}H NMR Data of Compounds **4**−**8** (in DMSO-d_6)

	δ [mult. J (Hz)]				
no.	**4**	**5**	**6**	**7***	**8**[a]
2	6.37 (d, 1.8)	6.39 (d, 1.8)	6.47 (d, 2.1)	6.49 (d, 2.1)	6.53 (d, 1.8)
4	6.36 (d, 1.8)	6.36 (d, 1.8)	6.73 (d, 2.1)	6.50 (d, 2.1)	6.55 (d, 1.8)
7	7.62 (d, 8.4)	7.30 (s)			
8	7.11 (d, 8.4)		6.44 (d, 1.8)	6.44 (s)	
10		7.55 (s)	6.98 (d, 1.8)		6.87 (s)
1-OCH$_3$					
3-OCH$_3$			3.83 (s)		
8-OCH$_3$		3.84 (s)			
9-OCH$_3$	3.86 (s)	3.84 (s)	3.80 (s)	3.85 (3s)	3.91 (s)
OH	10.90 (s)	10.90 (s)	11.15 (s)		9.31 (s)
	10.48 (s)	10.46 (s)	9.34 (s)		9.62 (s)
isoprenyl 1′	3.56 (d, 7.0)			3.47 (d, 7.5)	3.38 (d, 7.2)
2′	5.29 (t, 7.0)			5.26 (t, 7.5)	5.23 (m)
4′	1.83 (s)			1.80 (s)	1.78 (s)
5′	1.63 (s)			1.60 (s)	1.63 (s)

[a] Solvent: CD_3COCD_3.

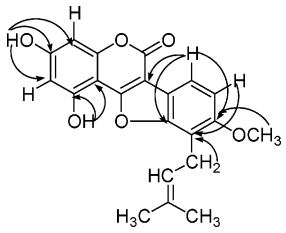

Figure 4. HMBC of **4**.

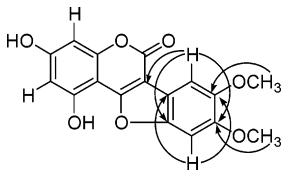

Figure 5. HMBC of **5**.

that the two hydroxyl groups were attached to C-1 and C-3 of the A-ring, respectively. In the HMBC spectrum, the *O*-methyl protons at δ 3.86 correlated with C-9 (δ 155.7) and the proton at δ 3.56 (H-1′) correlated with C-10 (δ 113.8), indicating that the *O*-methyl group and the isoprenyl group were attached to C-9 and C-10 of the B-ring, respectively. The key HMBC correlations are shown in Figure 4. All the signals in the ^{1}H NMR and ^{13}C NMR spectra were assigned according to detailed analysis of HSQC and HMBC spectra (Tables 1 and 3). The structure of **4** was thus elucidated as 1,3-dihydroxy-9-methoxy-10-γ,γ-dimethyllallylcoumestan, named hedysarimcoumestan D.

Hedysarimcoumestan E (**5**) was obtained as pale yellow needles, and its molecular formula $C_{17}H_{12}O_7$ was established by HRSI-MS at *m*/*z* 327.0506 [M − H]$^-$ (calcd 327.0510). The IR and UV spectroscopic data indicated that the structure of **5** was similar to that of **2**. The ^{1}H NMR spectrum of **5** displayed four aromatic protons of the A- and B-rings at δ 6.39 (1H, d, J = 1.8 Hz), 6.36 (1H, d, J = 1.8 Hz), 7.30 (1H, s), and 7.55 (1H, s). The signal at δ 3.84 (6H, s) was assigned to two *O*-methyl groups, and signals at δ 10.90 (1H, s) and 10.46 (1H, s) were assigned to two hydroxyl groups. The ^{13}C NMR spectrum showed 17 carbon signals. The ^{1}H and ^{13}C NMR spectra showed that there was one more *O*-methyl group in **5** than in **2**. On the basis of the HMBC correlations (Figure 5), the resonances at δ 6.39 and 6.36 were attributed to H-2 and H-4 of the A-ring, which generally resonate at a higher field, the resonances at δ 7.30 and 7.55 were attributed to H-7 and H-10 of the B-ring, which generally resonate at a lower field, and the two *O*-methyl groups were attached to C-8 and C-9 in the B-ring, respectively. All the signals in the ^{1}H NMR and ^{13}C NMR spectra were assigned on the basis of detailed analysis of HSQC and HMBC spectra (Tables 1 and 3). The structure of **5** was thus elucidated as 1,3-dihydroxy-8,9-dimethoxycoumestan, named hedysarimcoumestan E.

Hedysarimcoumestan F (**6**) was obtained as white needles, and its molecular formula $C_{17}H_{12}O_7$ was established by HRSI-MS at *m*/*z* 327.0509 [M − H]$^-$ (calcd 327.0510). The IR and UV spectroscopic data indicated that the structure of **6** was similar to that of **1**. The ^{1}H NMR spectrum of **6** showed four *meta*-coupled aromatic protons at δ 6.47 (1H, d, J = 2.1 Hz), 6.73 (1H, d, J = 2.1 Hz), 6.44 (1H, d, J = 1.8 Hz), and 6.98 (1H, d, J = 1.8 Hz). Two *O*-methyl protons at δ 3.83 (3H, s) and 3.80 (3H, s) and two hydroxyl protons at δ 11.15 (1H, s) and 9.34 (1H, s) were evident. Further, the ^{13}C NMR spectrum showed 17 carbon signals. The ^{1}H and ^{13}C NMR spectra of **6** showed that there was one more hydroxyl group in **6** than in **1**. The ^{1}H and ^{13}C NMR data of **6** for the A-ring were in good agreement with those of **1**, indicating that an *O*-methyl group and a hydroxyl group were attached to C-3 and C-1, respectively. This deduction was supported by the HMBC spectrum. The positions of the other hydroxyl group and the *O*-methyl group were determined by the HMBC spectrum. In the HMBC spectrum, the hydroxyl proton at δ 9.34 correlated with three carbons at δ 101.9 (C-6a), 104.3 (C-6b), and 150.5 (C-7), the *O*-methyl protons at δ 3.80 correlated with C-9 at δ 160.5, an aromatic proton at δ 6.44 (H-8) correlated with carbons at δ 150.5 (C-7) and 160.5 (C-9), and the other aromatic proton at δ 6.98 (H-10) correlated with carbons at δ 160.5 (C-9), 156.8 (C-10a), and 104.3 (C-6b), indicating that the hydroxyl group and the *O*-methyl group were attached to C-7 and C-9, respectively. The structure of **6** was thus elucidated as 1,7-dihydroxy-3,9-dimethoxycoumestan, named hedysarimcoumestan F.

Hedysarimcoumestan G (**7**) was obtained as pale yellow needles, and its molecular formula ($C_{21}H_{18}O_7$) was established by HRSI-MS at *m*/*z* 381.0980 [M − H]$^-$ (calcd 381.0980). The IR and UV spectroscopic data indicated that the structure of **7** was similar to that of **1**. The ^{1}H NMR spectrum displayed two *meta*-coupled aromatic protons at δ 6.49 (1H, d, J = 2.1 Hz) and 6.50 (1H, d, J = 2.1 Hz), belonging to a 1,2,3,5-tetrasubstituted aromatic ring, and an aromatic proton at δ 6.44 (1H, s) corresponding to a pentasubstituted aromatic system. The protons at δ 1.80 (3H, s), 1.60 (3H, s), 3.47 (2H, d, J = 7.5 Hz), and 5.26 (1H, t, J = 7.5 Hz) indicated the presence of an isoprenyl group. The signal at δ 3.85 (3H, s) was assigned to an *O*-methyl group. In the ^{13}C NMR spectrum of **7**, the resonances for the tetrasubstituted aromatic ring (C-1−C-4, C-4a, and C-11b) were in good agreement with those of **4** and **5**, indicating that **7** had a 1,3-dihydroxy-substituted A-ring. On comparison of the ^{13}C NMR data of the B-ring of **7** and **6**, the carbon at δ 105.6 (C-10) had a downfield shift of 16.3 ppm and the carbons at δ 148.6 (C-7), 95.4 (C-8), 157.7 (C-9), and 155.5 (C-10a) had upfield shifts of 1.3−3.1 ppm, respectively, indicating

that the isoprenyl group was attached to C-10. In the HMBC spectrum, the *O*-methyl protons at δ 3.85 correlated with C-9 (δ 157.7), the aromatic proton at δ 6.44 (H-8) correlated with carbons at δ 157.7 (C-9), 148.6 (C-7), 105.6 (C-10), and 103.7 (C-6b), and the isoprenyl proton at δ 3.47 (H-1′) correlated with carbons at δ 157.7 (C-9), 155.5 (C-10a), and 105.6 (C-10), indicating that the *O*-methyl group and the isoprenyl group were attached to C-9 and C-10, respectively. All the ^{1}H NMR and ^{13}C NMR data were assigned (Tables 1 and 3) on the basis of detailed analysis of HSQC and HMBC spectra. The structure of **7** was thus elucidated as 1,3,7-trihydroxy-9-methoxy-10-γ,γ-dimethyllallylcoumestan, named hedysarimcoumestan G.

Hedysarimcoumestan H (**8**) was obtained as white needles, and its molecular formula $C_{21}H_{18}O_7$ was established by HRSI-MS at *m*/*z* 381.0983 [M − H]$^-$ (calcd 381.0980). The IR and UV spectroscopic data indicated that the structure of **8** was similar to that of **7**. The ^{1}H NMR spectrum of **8** showed patterns similar to those of **7**. In the ^{13}C NMR spectrum of **8**, the signals were in good agreement with those of **7**, except that the carbon of C-8 in **8** had a downfield shift of 17.3 ppm and the carbon of C-10 had an upfield shift of 18.2 ppm, indicating that the isoprenyl group was attached to C-8 in **8**. In the HMBC spectrum, the aromatic proton at δ 6.87 (H-10) correlated with carbons at δ 158.8 (C-9), 155.2 (C-10a), 112.7 (C-8), and 104.4 (C-6b) and the aromatic proton at δ 3.38 (H-1′) correlated with C-8 (δ 112.7), thus supporting the above deduction. All the ^{1}H and ^{13}C NMR data were assigned (Tables 1 and 3) on the basis of detailed analysis of HSQC and HMBC spectra. The structure of **8** was thus elucidated as 1,3,7-trihydroxy-9-methoxy-8-γ,γ-dimethyllallylcoumestan, named hedysarimcoumestan H.

The structures of **9** and **10** were elucidated as 1,3,9-trimethoxycoumestan[6] and aureol,[7] respectively, on the basis of the analysis of UV, ^{1}H NMR, ^{13}C NMR, HSQC, and HMBC data. Although it had been synthesized,[6] it is the first isolation of 1,3,9-trimethoxycoumestan from a plant source.

Experimental Section

General Experimental Procedures. Melting points were determined on an X_4-A micro-melting point apparatus and are uncorrected. UV and IR spectra were recorded on a Shimadzu UV-260 spectrometer and a Perkin-Elmer 983 or Nicolet FT-5DX infrared spectrometer, respectively. ^{1}H NMR, ^{13}C NMR, HSQC, and HMBC spectra were measured on a Bruker DRX-500 spectrometer, operating at a basic frequency of 500 MHz. High-resolution secondary ionization mass spectrometry (HRSI-MS) was obtained on an APEX II mass spectrometer. D101 resin was purchased from Tianjin Chemical Co. Column chromatography silica gel (200−300 mesh) was a Qingdao Marine Chemical Factory product. Sephadex LH-20 was a Pharmacia product.

Plant Material. The roots of *H. multijugum* were collected from Yongdeng, Gansu Province, People's Republic of China, in July 1999. The plant was identified by one of the authors (H.-B.C.). A voucher specimen (99-W-013) was deposited in the Herbarium of the Department of Natural Medicines, School of Pharmaceutical Sciences, Peking University Health Science Center, People's Republic of China (PEM).

Extraction and Isolation. The air-dried powders of the roots of *H. multijugum* (8.0 kg) were percolated with 95% aqueous EtOH. After evaporation of the solvent under reduced pressure, the residues were suspended in H_2O and extracted successively with petroleum ether, EtOAc, and *n*-BuOH, respectively. Each solvent was evaporated under vacuum to yield the petroleum ether extract (35.0 g), EtOAc extract (650.0 g), *n*-BuOH extract (165.0 g), and a water-soluble material.

A portion of the EtOAc extract (300.0 g) was subjected to silica gel column chromatography with petroleum ether−acetone (100:0 → 0:100) to give 10 fractions. Fraction 7 (11.8 g) was chromatographed on a Sephadex LH-20 column, eluted with MeOH, to yield **1** (11 mg). Fraction 4 (20.9 g) was chromatographed on a silica gel column, eluted with petroleum ether−$CHCl_3$ (1:1), to yield **9** (10 mg). Fraction 9 (23.5 g) was subjected repeatedly to multiple silica gel column separations using petroleum ether−acetone (5:1) and petroleum ether−$CHCl_3$ (2:1) as eluent, respectively, then subjected to an Rp-18 column using acetone as eluent to yield **4** (11 mg), **5** (8 mg), and **8** (16 mg). Fraction 8 (30.4 g) was subjected repeatedly to silica gel column separations by using petroleum ether−$CHCl_3$ (1:1) as eluent and to Sephadex LH-20 column chromatography, eluted with 90% MeOH, to yield **6** (12 mg) and **7** (11 mg). Fraction 10 (28.5 g) was subjected repeatedly to silica gel column separations using petroleum ether−acetone (3:1) as eluent and to Sephadex LH-20 column chromatography, eluted with 90% MeOH, to yield **10** (15 mg). The *n*-BuOH extracts (165 g) were subjected to D101 aporous resin column chromatography, eluted consecutively with H_2O, 50% EtOH, 70% EtOH, and EtOH. The 50% EtOH-soluble material (27.8 g) was subjected to silica gel column chromatography with $CHCl_3$−MeOH (100:0 → 0:100) to give eight fractions. Fraction 3 (2.5 g) was chromatographed on an Rp-18 column, eluting with 20% MeOH, to yield **2** (10 mg). Fraction 6 (3.2 g) was chromatographed on an Rp-18 column eluting with 20% MeOH, followed by a Sephadex LH-20 column separation, eluted with 70% MeOH, to yield **3** (8 mg).

Hedysarimcoumestan A (1): purple needles (MeOH); mp >300 °C; UV (MeOH) λ_{max} 331, 262, 224 nm; IR (KBr) ν_{max} 3312, 2943, 2841, 2360, 2340, 1697, 1625, 1555, 1498, 1330, 1274, 1163, 1093, 993, 822, 667, 465 cm^{-1}; HRSI-MS (neg) *m*/*z* 311.0561 [M − H]$^-$ (calcd for $C_{17}H_{12}O_6$, 311.0567); ^{1}H NMR and ^{13}C NMR data, in Table 1 and Table 2.

Hedysarimcoumestan B (2): pale yellow needles (MeOH); mp >300 °C; UV (MeOH) λ_{max} 342, 267, 224 nm; IR (KBr) ν_{max} 3322, 3083, 2925, 2846, 2360, 2340, 1714, 1632, 1616, 1650, 1522, 1495, 1351, 1269, 1173, 1095, 1019, 975, 819, 727, 624, 561, 470 cm^{-1}; HRSI-MS (neg) *m*/*z* 297.0404 [M − H]$^-$ (calcd for $C_{16}H_{10}O_6$, 297.0405); ^{1}H NMR and ^{13}C NMR data, in Table 1 and Table 2.

Hedysarimcoumestan C (3): pale yellow needles (MeOH); mp >300 °C; UV (MeOH) λ_{max} 346, 256, 224 nm; IR (KBr) ν_{max} 3296, 2925, 2360, 2340, 1654, 1605, 1510, 1443, 1360, 1301, 1205, 1065, 820, 793, 653, 595 cm^{-1}; HRSI-MS (neg) *m*/*z*: 445.0770 [M − H]$^-$ (calcd for $C_{21}H_{18}O_{11}$, 445.0776); ^{1}H NMR and ^{13}C NMR data, in Table 1 and Table 2.

Hedysarimcoumestan D (4): pale yellow needles (MeOH); mp 270−275 °C; UV (MeOH) λ_{max} 345, 258, 224 nm; IR (KBr) ν_{max} 3374, 2925, 2846, 2360, 2340, 1711, 1628, 1572, 1499, 1338, 1262, 1152, 1064, 953, 840, 669, 421 cm^{-1}; HRSI-MS (neg) *m*/*z*: 365.1029 [M − H]$^-$ (calcd for $C_{21}H_{18}O_6$, 365.1031); ^{1}H NMR and ^{13}C NMR data, in Table 1 and Table 3.

Hedysarimcoumestan E (5): pale yellow needles (MeOH); mp >300 °C; UV (MeOH) λ_{max} 346, 299, 224 nm; IR (KBr) ν_{max} 3294, 2920, 2360, 2340, 1707, 1627, 1495, 1336, 1275, 1155, 1091, 999, 898, 833, 669 cm^{-1}; HRSI-MS (neg) *m*/*z* 327.0506 [M − H]$^-$ (calcd for $C_{17}H_{12}O_7$, 327.0510); ^{1}H NMR and ^{13}C NMR data, in Table 1 and Table 3.

Hedysarimcoumestan F (6): white needles (MeOH); mp >300 °C; UV (MeOH) λ_{max} 348, 265, 224 nm; IR (KBr) ν_{max} 3361, 2929, 2840, 2360, 2340, 1682, 1628, 1506, 1422, 1316, 1150, 1075, 962, 805, 669, 470 cm^{-1}; HRSI-MS (neg) *m*/*z* 327.0509 [M − H]$^-$ (calcd for $C_{17}H_{12}O_7$, 327.0510); ^{1}H NMR and ^{13}C NMR data, in Table 1 and Table 3.

Hedysarimcoumestan G (7): pale yellow needles (MeOH); mp >300 °C; UV (MeOH) λ_{max} 359, 274, 224 nm; IR (KBr) ν_{max} 3324, 2924, 2846, 2360, 2340, 1672, 1630, 1571, 1432, 1382, 1290, 1151, 1058, 827, 669, 419 cm^{-1}; HRSI-MS (neg) *m*/*z* 381.0980 [M − H]$^-$ (calcd for $C_{21}H_{18}O_7$, 381.0980); ^{1}H NMR and ^{13}C NMR data, in Table 1 and Table 3.

Hedysarimcoumestan H (8): white needles (MeOH); mp >300 °C; UV (MeOH) λ_{max} 353, 269, 224 nm; IR (KBr) ν_{max} 3319, 2919, 2360, 2340, 1680, 1631, 1568, 1419, 1325, 1233, 1158, 1063, 939, 833, 771, 635, 553, 472 cm^{-1}; HRSI-MS (neg) *m*/*z* 381.0983 [M − H]$^-$ (calcd for $C_{21}H_{18}O_7$, 381.0980); ^{1}H NMR and ^{13}C NMR data, in Table 1 and Table 3.

1,3,9-Trimethoxycoumestan (9): white needles (MeOH); mp 220−225 °C; UV (MeOH) λ_{max} 343, 250, 225 nm; IR (KBr) ν_{max} 3424, 2945, 2842, 2360, 2340, 1745, 1611, 1500, 1461, 1374, 1271, 1157, 1089, 1048, 961, 828 cm^{-1}; HRSI-MS (pos) *m*/*z* 327.0866 [M + H]$^+$ (calcd for $C_{18}H_{14}O_6$, 327.0863); ^{1}H NMR and ^{13}C NMR data, in Table 1 and Table 2.

Aureol (10): pale yellow needles (MeOH); mp >300 °C; UV (MeOH) λ_{max} 344, 264, 228 nm; IR (KBr) ν_{max} 3311, 3103, 2924, 2360, 2340, 1705, 1627, 1520, 1446, 1381, 1301, 1264, 1157, 1069, 979, 815, 721, 627, 565 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, in Table 1 and Table 2.

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References and Notes

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