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Phytochemical studies of *Rehmannia glutinosa* rhizomes

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2,4-Dimethoxy-2-methyl-6H-pyran-3-one (**1**), a hitherto unknown natural product, and the calcium salt of rehmapicroside (**2**) have been isolated from rhizomes of the Vietnamese variety of *Rehmannia glutinosa* Libosch together with a series of known compounds: norcarotenoids (**3–5**), 2-formyl-5-hydroxymethylfurane (**6**), the iridoid rehmaglutin D (**7**), iridoid glycosides (**8–12**) and phenylethyl alcohol glycosides (**13–17**). Their structures were determined by mass and NMR spectroscopy.

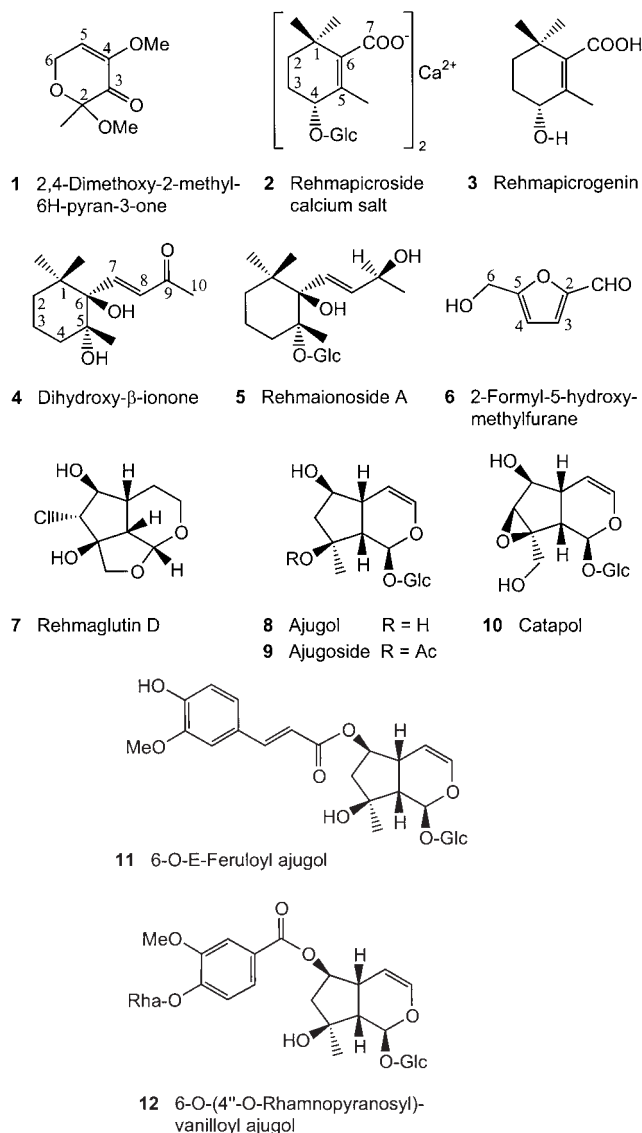
1. Introduction

The fresh, dried, or steamed rhizomes of *Rehmannia glutinosa* (Scrophulariaceae) represent one of the most important drugs in the Eastern herbal medicine used as tonic, antianemic, antipyretic and antihypoglycemic [1]. In a Vietnamese traditional medicine book [2] rhizomes of *R. glutinosa* have been described as a blood glucose decreasing agent, active with the central nervous system, diuretic and antiviral. Until now, several chemical investigations of Japanese, Chinese, and Korean *R. glutinosa* have been carried out to discover many iridoids, iridoid glycosides [3, 4], norcarotenoids [5], norcarotenoid glycosides [6], phenethyl alcohol glycosides [7] as well as various carbohydrates and amino acids [8]. However, no studies on the chemical constituents of the *Rehmannia glutinosa* variety growing in Vietnam have been reported. In this paper we describe the results of the phytochemical investigation of the dried rhizomes of *R. glutinosa* growing in Northern Vietnam. From the twenty isolated compounds 2,4-dimethoxy-2-methyl-6H-pyran-3-one (**1**) is unknown as natural product and the calcium salt of the norcarotenoid glycoside rehmapicroside (**2**) is a new compound. The isolation and structural elucidation of **1** and **2** as well as the four known compounds rehmapicrogenin (**3**), rehmaionoside A (**4**), dihydroxy- β -ionone (**5**) and 2-formyl-5-hydroxy-methylfurane (**6**) are discussed in detail.

2. Investigations, results and discussion

Chromatographic separation of the ethyl acetate and methanol extracts of rhizomes of *R. glutinosa* afforded 20 compounds.

The ESI-MS spectrum of the amorphous compound **1** displayed the base peak at m/z 195 $[M + Na]^+$. The ¹H- and ¹³C NMR spectra exhibited the existence of one tertiary methyl group (δ_H 1.51 s, δ_C 17.8), two methoxy groups (δ_H 3.37 s, 3.64 s; δ_C 50.1, 54.9), two geminal protons [4.58 (dd, $J = 17.3, 2.2$ Hz) and 4.32 (dd, $J = 17.3,$



4.7 Hz)] and one olefinic proton [5.82 (dd, $J = 4.7$, 2.2 Hz)]. On the basis of these data the structure of **1** was determined as 2,4-dimethoxy-2-methyl-6H-pyran-3-one, found for the first time as a natural product. The spectral data of **1** are in good agreement with those of a synthesized compound [9].

The spectroscopic analysis of the amorphous compound **2** revealed that it is a glucoside with one vinylic methyl group (δ_{H} 1.78 s, δ_{C} 18.7), two tertiary methyl groups [δ_{H} 1.14 s (6H); δ_{C} 28.2, 29.6] and one α,β -unsaturated carboxylate ion (δ_{C} 180.6). Detailed comparison of the ^1H - and ^{13}C NMR data with those of rehmapicroside and the known calcium salt of rehmapicrogenin [5, 10] suggested that **2** has the structure of rehmapicroside, but as a metal salt. By atomic absorption spectroscopy (AAS) the metal ion in **2** has been determined as calcium. This is the first time, the calcium salt of rehmapicroside (**2**) was isolated from plant material.

Comparison of the spectral data of **3** with those of compound **2** indicated that **3** is the aglycone of rehmapicroside, named rehmapicrogenin. The NMR spectral data of **3** are in good agreement with that published for rehmapicrogenin [5].

Compound **4** was isolated as an amorphous powder. Its EI-MS spectrum gave a molecular ion peak at m/z 226 $[\text{M}]^+$. The ^1H NMR spectrum of **4** exhibited *trans*-olefinic proton signals at δ 6.35 and 7.33 (each 1H, d, $J = 16.2$ Hz) and a methyl adjacent to the carbonyl group at δ 2.32 (3H, s) indicating the existence of a 3*E*-buten-2-onyl side chain. The ^1H NMR spectrum exhibited three further methyl singlets at δ 0.83, 1.13 and 1.23 (each 3H) whereas the ^{13}C NMR showed two signals for quaternary carbons carrying a hydroxyl group at δ 74.9 and 79.4. This suggested that compound **4** has a 5,6-dihydroxy-1,1,5-trimethylcyclohexyl structure. This was finally confirmed by comparison with published data [5] of the known dihydroxy- β -ionone (**4**).

Compound **5** also was obtained as an amorphous powder. Comparison of the ^1H - and ^{13}C NMR data of **5** with those of dihydroxy- β -ionone (**4**) suggested that **5** contains a glucose bound to the 5,6-dihydroxy-1,1,5-trimethylcyclohexyl

moiety, especially indicated by the NMR signals at δ_{H} 4.44 (d, 7.7 Hz); δ_{C} 98.1 for an anomeric proton and C-1 of the sugar residue. The NMR signal of the aglycone C-5 carbon is shifted to lower field by 8.7 ppm in **5**, showing that the glucose unit is linked at C-5. The ^1H NMR spectrum of **5** exhibited *trans*-olefinic proton signals at δ 5.68 (1H, dd, $J = 15.9$, 6.5 Hz) and 6.22 (1H, dd, $J = 15.9$, 1.1 Hz), and a methyl adjacent to a hydroxylated methine group at δ 1.25 (3H, d, $J = 6.6$), which showed the existence of a 3-hydroxy-*E*-butenyl side chain. Thus, the NMR data suggested that compound **5** is identical with rehmapicroside A, which was finally confirmed by comparison with the spectral data published in the literature [6]. NMR data of compounds **2**–**5** are given in the Table.

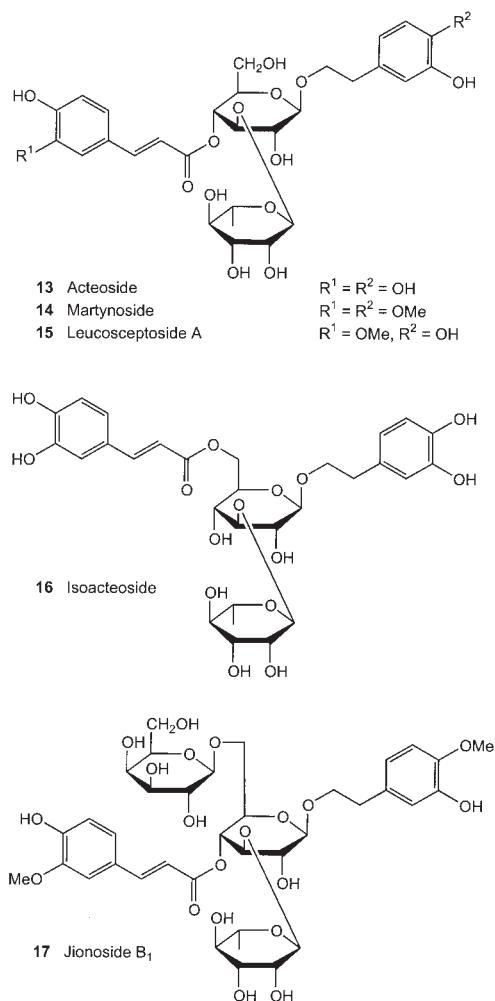
Compound **6** was isolated as oil. The EI-MS spectrum showed the molecular ion peak at m/z 126 $[\text{M}]^+$. The ^1H - and ^{13}C NMR spectra of **6** (see Experimental) showed that the molecule contains one aldehyde group (δ_{H} 9.60 s, δ_{C} 177.4), a hydroxylated methylene group (δ_{H} 4.73 s, δ_{C} 57.7) and two proton signals of a furane ring [δ_{H} 7.22, 6.53 (each 1H, d, $J = 3.58$ Hz) and δ_{C} 122.5, 109.9] characterizing compound **6** as 2-formyl-5-hydroxymethyl-furane.

Furthermore the known compounds rehmaglutine D (**7**), ajugol (**8**), ajugoside (**9**), catalpol (**10**), 6-*O*-*E*-feruloyl ajugol (**11**), 6-*O*-(4''-*O*- α -L-rhamnopyranosyl)vanilloyl ajugol (**12**), acteoside (**13**), martynoside (**14**), leucosceptoside A (**15**), isoacteoside (**16**), jionoside B₁ (**17**) as well as the common compounds adenosin, uridin and n-butyl fructofuranose were isolated. All known compounds except **6** were also reported from *R. glutinosa* from other countries. Their structures were determined by comparison with spectroscopic reference data.

Although the constituents of *Rehmannia glutinosa* and their biological activities have been studied intensively, the relationship between the components and the pharmacological activities of the drug remains obscure [11]. However, several activities of its components were found. For example its phenolic glycosides showed immunosuppressive and antibiotic activities against various fungi [12, 13]. The norcarotenoid jiocarotenoside A1 possesses an

Table: ^{13}C and ^1H NMR-Data of compounds **2**, **3**, **4** and **5**

position	2 (CD ₃ OD)		3 (CD ₃ OD)		4 (CDCl ₃)		5 (CD ₃ OD)	
	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}
1	33.8	—	34.3	—	38.5	—	39.3	—
2	35.2	1.32 m	35.7	1.42 m	36.3	1.30 m	37.2	1.13 m
3	25.4	1.68–1.78 m	29.5	1.60–1.76 m 1.92 m	17.8	1.4–1.6 m	18.7	1.6–1.8 m
4	75.0	4.09 t (4.0)	69.3	3.92 t (5.5)	36.3	1.6–1.9 m	32.6	2.10 m
5	126.0	—	133.9	—	74.9	—	83.6	—
6	146.2	—	139.8	—	79.4	—	79.4	—
7	180.6	—	173.7	—	149.2	6.35 d (16.2)	134.5	6.22 dd (15.9, 1.1)
8	—	—	—	—	130.6	7.33 d (16.2)	132.4	5.68 dd (15.9, 6.5)
9	—	—	—	—	198.1	—	69.7	4.33 br qui (6.3)
10	—	—	—	—	25.1	2.32 s	24.3	1.25 d (6.6)
Me ₂ -1	28.2	1.14 s	28.1	1.09 s	26.5	0.83 s	25.9	0.81 s
	29.6	1.14 s	28.7	1.13 s	27.4	1.23 s	27.6	1.16 s
Me-5	18.7	1.78 s	18.1	1.77	27.6	1.13 s	22.4	1.19 s
1'	101.5	4.34 d (7.7)	—	—	—	—	98.1	4.44 d (7.7)
2'	75.0	—	—	—	—	—	75.5	—
3'	78.1	H-2'-H-5':	—	—	—	—	79.1	H-2-H-5':
4'	71.9	3.1–3.4 m	—	—	—	—	71.8	3.2–3.4 m
5'	77.8	—	—	—	—	—	77.3	—
6'	62.9	3.67 dd (11.8, 5.4) 3.86 dd (11.8, 1.9)	—	—	—	—	62.8	3.61 dd (11.8, 5.8) 3.81 dd (11.8, 2.3)



inhibitory effect against aldose reductase [6]. It was supposed that rehmanin is responsible for lowering the blood sugar levels [2].

3. Experimental

3.1. Instruments

NMR: Varian Gemini 300, 300.24 MHz (¹H) and 75.5 MHz (¹³C). Chemical shifts were referenced to internal TMS ($\delta = 0$, ¹H) and CDCl₃ ($\delta = 77.0$, ¹³C) or CD₃OD ($\delta = 49.0$, ¹³C). EIMS: AMD 402, 70 eV. $[\alpha]_D$: Digital Polarimeter Jasco Tip 1000. CC: silica gel (Merck, 40–63 μm), Sephadex LH 20 (Fluka), LiChroprep RP 18 (Merck, 40–63 μm). Preparative TLC: precoated silica gel plates 60 F₂₅₄, thickness 0.5 or 1 mm.

3.2. Plant material

The roots of *Rehmannia glutinosa* Libosch were bought in the market for medicinal plants in Hanoi, July, 2000. The plant species was confirmed by Mr. Ngo Van Trai, Institute of Materia Medica, Hanoi. A voucher specimen was deposited in the herbarium of this institute.

3.3. Extraction and isolation

Rhizomes of *Rehmannia glutinosa* Libosch were dried, ground and extracted with hot 95% EtOH. The organic solvent was evaporated *in vacuo* and the aqueous solution extracted successively with *n*-hexane, EtOAc and *n*-BuOH. EtOAc was removed *in vacuo* and the residue was chromatographed over silica gel with CHCl₃ and increasing amounts of MeOH. From fractions eluted with 5% MeOH compounds **1**, **4** and **6** were isolated.

The fraction containing compound **1** was purified by CC on Sephadex LH 20 (MeOH) and silica gel (CHCl₃–MeOH 95:5), followed by preparative TLC (CHCl₃–MeOH 90:10) to give **1** (8 mg), an amorphous compound. EI-MS *m/z* (rel. int.): 142 (21.4), 101 (15.0), 70 (38.6), 55 (100). ¹H NMR (CDCl₃): δ 1.51 (3 H, s, Me-2), 3.37, 3.64 (each 3 H, s, 2-OMe, 4-OMe), 4.32 (1 H, dd, $J = 17.3$, 4.7 Hz, H-6), 4.58 (1 H, dd, $J = 17.3$, 2.2 Hz, H-6), 5.82 (1 H, dd, $J = 4.7$, 2.2 Hz, H-5). ¹³C NMR (CDCl₃): δ 17.8 (Me-2), 50.1, 54.9 (2-OMe and 4-OMe), 59.4 (C-6), 99.5 (C-2), 113.0 (C-5), 146.5 (C-4), 186.2 (C-3).

The fraction containing **4** was given over a flash silica gel column eluting with *n*-hexane–acetone (7:3). The following purification was carried out by preparative TLC (CHCl₃–MeOH 95:5) to yield compound **4** (10 mg). $[\alpha]_D^{25} = -38.6^\circ$ (c 0.44, MeOH). EI-MS *m/z* (rel. int.): 226 [M]⁺ (0.9), 208 (5), 165 (15.7), 141 (18.6), 123 (60.7), 109 (100), 99 (35.7), 85 (16.4), 71 (36.0). ¹H and ¹³C NMR: see Table.

The fraction containing **6** was subjected to a flash silica gel column eluting with *n*-hexane–acetone (1:1). Further purification by preparative TLC developed with CHCl₃–MeOH (90:10) gave compound **6** (6 mg). EI-MS *m/z* (rel. int.): 126 [M]⁺ (100), 107 (51.4), 97 (62.8), 53 (43.6). ¹H NMR (CDCl₃): δ 4.73 (2 H, s, H-6), 6.53 (1 H, d, $J = 3.58$ Hz, H-4), 7.22 (1 H, d, $J = 3.58$ Hz, H-3), 9.60 (1 H, s, 2-CHO). ¹³C NMR (CDCl₃): δ 57.7 (5-CH₂OH), 109.9 (C-4), 122.5 (C-3), 152.3 (C-5), 160.3 (C-2), 177.4 (2-CHO).

With 20% MeOH the fraction of **2** was obtained. This fraction was rechromatographed over Sephadex LH 20 CC eluting with MeOH and purified by flash CC on silica gel with CHCl₃–MeOH–H₂O (70:25:2) to give **2** (20 mg). $[\alpha]_D^{25} = -9.9^\circ$ (c 1.39, MeOH). ¹H- and ¹³C NMR: see Table.

Elution with 10% MeOH yielded the fraction containing **3**. This fraction was rechromatographed over a Sephadex column eluting with MeOH and over a flash silica gel column with CHCl₃–MeOH (8:2) to isolate compound **3** (9 mg). $[\alpha]_D^{25} = 0^\circ$ (c 0.58, MeOH) {[1]: $[\alpha]_D^{24} = 0^\circ$ (c 0.27, MeOH)}. EIMS *m/z* (rel. int.): 184 [M]⁺ (2.9), 169 (3.6), 151 (4.3), 136 (100), 110 (33.6). ¹H- and ¹³C NMR: see Table.

The BuOH extract was chromatographed over silica gel with CHCl₃ and increasing amounts of MeOH. The fraction containing compound **5** was eluted with 25% MeOH. Further separation by CC on Sephadex LH 20 (MeOH), silica gel (CHCl₃–MeOH 85:15) and RP18 (MeOH–H₂O 3:7) afforded compound **5** (14 mg), an amorphous powder. $[\alpha]_D^{25} = -76.7^\circ$ (c 0.83, MeOH) {[2]: $[\alpha]_D^{25} = -73.6^\circ$ (c 0.49, MeOH)}. EIMS *m/z* (rel. int.): 228 [M–glucose]⁺ (1.8), 210 (18.6), 193 (23.6), 64 (100), 57 (22.8). ¹H- and ¹³C NMR: see Table.

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