Chinese Chemical Letters Vol. 11, No. 10, pp. 897–900, 2000

Two New Glycosides from *Rubus amabilis*

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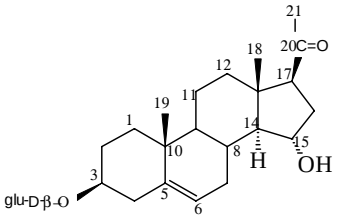
Abstract: A pregnane glycoside and a lignan glycoside were isolated from the aerial parts of *Rubus amabilis*. Their structures were elucidated as 3-O-β-D-glucopyranosyl-3β,15α-dihydroxypregn-5-en-20-one and (-)-secoisolariciresinol-O-α-L-rhamnopyranoside using spectroscopic and chemical methods.

Keywords: *Rubus amabilis*, Rosaceae, pregnane, lignan, glycoside.

Rubus amabilis Focke is a shrub which widely spread in China. Its fruits are often processed into health drinks. Its roots, branches and leaves have fairly good medical values[1]. No phytochemical examination on the plant has been reported up to now. Two new glycosides were obtained from this plant. To our knowledge, they are the first C_{21}-steroid and the first lignan isolated from the genus *Rubus*.



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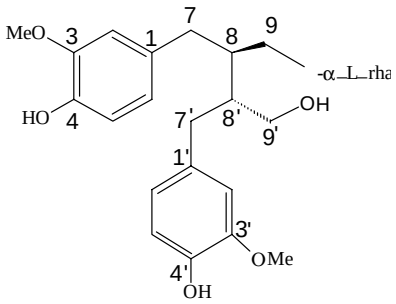
Compound **1** was obtained as a white amorphous powder, mp: 290-292°C, $[\alpha]_D^{20}$ +110 (c 0.25, pyridine). Its IR spectrum (KBr) revealed the presence of hydroxyl (3434cm^{-1}), carbonyl (1696cm^{-1}), and a glycosidic linkage (1073cm^{-1}). The FAB mass spectrum displayed a quasi-molecular ion peak at *m/z* 517 $[M+Na]^+$ and a prominent fragment ion peak at *m/z* 315 $[M-Glu]^+$ due to the loss of sugar moiety. In combination with the ^{13}CNMR and DEPT spectral data (**Table 1**), the molecular formula of **1** was determined to be $C_{27}H_{42}O_8$. The ^{1}H, ^{13}CNMR and DEPT spectra showed signals for a glucose unit which was confirmed by PC after acid hydrolysis of **1**, as well as for a characteristic skeleton of C_{21}-steroid: three methyls, seven methylenes, seven methines (including two oxygenated methines), three quaternary carbons and one carbonyl.

Comparison of the ^{1}H, ^{13}CNMR and DEPT spectral data of **1** with those of a similar compound, Carumbelloside II[2], showed that the aglycone of **1** was dihydroxypregn-5-en-20-one. In the ^{1}H-^{1}H COSY spectrum the proton at δ 3.46 correlated with H-2 (δ 1.81, 1.46) and H-4 (δ 2.38, 2.12), and the proton at δ 4.04 correlated with H-14 (δ 0.93) and H-16 (δ 2.00). This indicated that the two hydroxyl groups were at C-3 and C-15 respectively. In steroids, if C/D rings were in the *cis*-configuration, Me-18 proton signal was at about δ 1.0; if in the *trans*-configuration, at about δ 0.7[3]. The Me-18 signal of **1** was at δ 0.73, therefore the C/D rings were in *trans*-configuration, and H-14 was in α-configuration. In the NOESY spectrum, absence of NOE cross peaks between H-14α and H-15 suggested that H-15 was in β-configuration and the hydroxyl group was in α-configuration. Presence of NOE cross peaks between H-14α and H-17 showed that the acetyl was in β-configuration. The linkage position of the sugar unit was established by the HMBC experiment. The cross peaks between H-3 (δ 3.46) and C-1 of Glu (δ 100.8), and between C-3 (δ 76.7) and H-1 of Glu (δ 4.21) were observed. Moreover the anomeric proton signal at δ 4.21 (1H, d, J=7.6Hz) in the ^{1}HNMR spectrum indicated a β-configuration for the glucosyl moiety. In conclusion, the structure of **1** was clarified as 3-O-β-D-glucopyranosyl-3β,15α-dihydroxypregn-5-en-20-one. Its ^{1}H and ^{13}CNMR data were assigned by the use of HMQC and HMBC experiments.

Table 1. ^{1}HNMR (400 MHz), ^{13}CNMR (100 MHz) and DEPT data of **1** (DMSO-d_6,TMS, δ, ppm)

NO	^{1}H, (α/β)	^{13}C	DEPT	NO	^{1}H, (α/β)	^{13}C	DEPT
1	1.00(m)/1.81(br.d,11.0)	36.9	CH_2	15	-/4.04(br.)	68.1	CH
2	1.81(br.d,11.0)/1.46(m)	29.3	CH_2	16	2.00[a](m)/1.98[a](m)	35.5	CH_2
3	3.46(m)/-	76.7	CH	17	2.46(t,9.3)/-	63.0	CH
4	2.38(dd,12.5,4.0)/ 2.12(br.t,12.4)	38.3	CH_2	18	0.73(s)	15.4	CH_3
5	-	140.3	C	19	0.96(s)	19.0	CH_3
6	5.40(d,2.0)	121.3	CH	20	-	208.0	C
7	2.23(br.d,18.9)/1.51(m)	30.3	CH_2	21	2.04(s)	31.2	CH_3
8	-/1.72(m)	27.4	CH	Glu-1	4.21(d,7.6)	100.8	CH
9	0.95(m)/-	49.7	CH	2	2.88(br.t,7.9)	73.4	CH
10	-	36.3	C	3	3.11(br.t,8.2)	76.9	CH
11	1.55(m)/1.40(m)	20.6	CH_2	4	3.00(br.t,8.5)	70.1	CH
12	1.38(m)/1.93(br.d,6.2)	39.1	CH_2	5	3.05(m)	76.7	CH
13	-	43.0	C	6	3.40(dd,11.4,5.5), 3.64(br.d,11.4)	61.1	CH_2
14	0.91(dd,11.0,5.4)/-	60.5	CH				

Signal multiplicity and coupling constants (Hz) are in parentheses.
[a] interchangeable values.



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Compound **2** was obtained as colorless gum, $[\alpha]_D^{20}$ -49.5 (c 0.30, aceton). Its IR spectrum (film) indicated absorption bands for hydroxyl groups (3389cm^{-1}), phenyl rings (1604, 1516, 1450 cm^{-1}), C(sp^2)-O bonds (1271, 1235 cm^{-1}), C(sp^3)-O bonds (1152, 1126 cm^{-1}). ^{1}HNMR spectrum of **2** (**Table 2**) showed the presence of aromatic rings at δ 6.55-6.70, two methoxy groups at δ 3.74 (3H, s) and 3.75 (3H, s), and an anomeric proton of rhamnose at δ 4.62 (1H, d, J=1.2Hz) which indicated an α-configuration for the rhamnosyl moiety. Two sets of benzene rings, four methylenes (including two oxygenated methylenes), two methines and one set of α-L-rhamnopyranosyl signals were observed in its ^{13}CNMR and DEPT spectra (**Table 2**). Acid hydrolysis of **2** yielded the genin and L-rhamnose as the sugar moiety, which was detected by direct comparison

Table 2. ^{1}HNMR (400 MHz), ^{13}CNMR (100 MHz) and DEPT data of **2** (aceton-d_6,TMS, δ, ppm)

NO	^{1}H	^{13}C	DEPT	NO	^{1}H	^{13}C	DEPT
1	-	133.2	C	5'	6.70(d,8.0)	115.4	CH
2	6.68(d,1.6)	113.1	CH	6'	6.55(dd,8.0,1.6)	122.3	CH
3	-	148.1	C	7'	2.65(m)	35.3	CH_2
4	-	145.4	C	8'	1.97(m)	44.6	CH
5	6.70(d,8.0)	115.4	CH	9'	3.51(m),3.58(m)	62.0	CH_2
6	6.58(dd,8.0,1.6)	122.2	CH	3,3'-Ome	3.74(s),3.75(s)	56.1	CH_3
7	2.59(dd,13.6,8.4), 2.69(dd,13.6,6.8)	35.3	CH_2	Rha-1	4.62(d,1.2)	101.4	CH
8	2.12(m)	41.1	CH	2	3.88(d,3.2)	71.9	CH
9	3.35(m),3.69(m)	68.3	CH_2	3	3.66(m)	72.6	CH
1'	-	133.5	C	4	3.38(m)	73.6	CH
2'	6.66(d,1.6)	113.1	CH	5	3.54(m)	69.3	CH
3'	-	148.1	C	6	1.18(d,6.4)	18.1	CH_3
4'	-	145.4	C				

Signal multiplicity and coupling constants (Hz) are in parentheses.

with an authentic sample on PC. The EI mass spectrum of the genin displayed a molecular ion peak at *m/z* 362 $[M]^+$ and an ion peak at *m/z* 344 $[M-18]^+$, suggesting the molecular formula of the genin to be $C_{20}H_{26}O_6$, which was also supported by ^{13}CNMR and DEPT data. The above data suggested that **2** was a diphenylbutane-type lignan

rhamnoside with a molecular formula of $C_{26}H_{36}O_{10}$. The ^{13}CNMR signals of the aglycone portion corresponded closely with those reported for (-)-secoisolariciresinol-O-β-D-glucopyranoside[4]. ^{1}H-^{13}C correlation between H-1 of Rha and C-9 in the HMBC spectrum confirmed that L-rhamnose was connected at C-9 position. It follows that the structure of **2** is (-) secoisolariciresinol-O-α-L-rhamnopyranoside. Its ^{1}H and ^{13}CNMR data were assigned precisely by means of HMQC and HMBC experiments.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China NO.29972017, the Foundation of Ministry of Education of China for Doctoral Program NO.98073003 and National Key Basic Research Development Plan NO.G1998051113.

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Received 10 April 2000