Two Novel Tetrairidoid Glucosides from *Dipsacus Asper*

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Experimental

Extraction and isolation

The dried roots of *D. asper* (2 kg) were powdered and extracted three times with the boiling ethanol (10 L). The combined alcohol extracts were concentrated *in vacuo* to yield a dark brown residue (390 g). The ethanol extract was suspended in water and then partitioned in turn with petroleum ether, chloroform, ethyl acetate, and *n*-butanol. The butanol extract was evaporated under reduced pressure to yield 360 g of a residue. The residue was separated by column chromatography on macroporous resin D₁₀₁ with a EtOH : H₂O (0:100, 20:80, 40:60, 60:40, 80:20, 100:0) gradient system to give five fractions. Fraction 3 (30 g) was chromatographed over Sephadex LH-20 using (MeOH : H₂O, 80 : 20) to yield four subfractions. Subfraction 3-2 (10 g) was further separated by column chromatography over Rp-18 using MeOH : H₂O (10:90, 30:70, 40:60, 60:40, 0:100) gradient system to give six subfractions. Subfraction 3-2-3 (3.6 g), containing iridoid glucosides, was subjected to a series of purification steps using Sephadex LH-20 (MeOH : H₂O, 80 : 20) and Rp-18 (CH₃CN : H₂O, 20:80) column chromatography, preparative TLC (CHCl₃ : MeOH : H₂O, 75 : 25 : 10), and finally purified by preparative reversed phase HPLC on a C-18 column using CH₃CN : H₂O (20 : 80) as mobile phase to afford 60 mg of **1**, 8 mg of **2**.

HPLC analysis of *D. asper* extracts

The dried roots of *D. asper* (11 g) were powdered and divided into two equal sections. One section (5.1 g) was extracted with 95% EtOH at room temperature and gave the extract 1.683 g (A), while the other was extracted with boiling EtOH and afforded the extract 1.628 g (B). Both of the ethanol extracts were suspended in water and then partitioned in turn with petroleum ether, chloroform, ethyl acetate, and *n*-butanol, respectively. The butanol soluble part was evaporated under reduced pressure to yield the residue 0.6696 g (A-4) and 1.1921 g (B-4), respectively. A-4

and B-4 were resolved in 2 ml MeOH, respectively, and then detected by HPLC (Dima-ZY1104 ODS C-18, 150×4.6 mm; eluent, 20% CH₃CN; flow rate, 1.0 ml/min; detection UV at 240 nm). Compounds **1** and **2** can be recognized at 7.56 min and 9.63 min, respectively.

Table 1. ^1H - and ^{13}C - NMR spectral data for compound **1** in CD_3OD (500 MHz for ^1H and 125 MHz for ^{13}C)

^1H		^1H		^{13}C		^{13}C	
1a ₁	5.52 (4.0) ^P	1a ₂	5.43 (d, 5.5)	1a ₁	98.3	1a ₂	97.2
3a ₁	7.42 (s)	3a ₂	7.49 (s)	3a ₁	154.1	3a ₂	151.9
4a ₁		4a ₂		4a ₁	113.1	4a ₂	109.8
5a ₁	2.95 (br m)	5a ₂	4.05 (d, 5.5)	5a ₁	32.9	5a ₂	30.7
6a ₁	3.16 (dd, 8.0, 9.0) 2.33 (ddd, 6.0, 8.5) ^P	6a ₂		6a ₁	29.6	6a ₂	143.7
7a ₁	6.71 (dd, 6.5, 7.0)	7a ₂	9.26 (s)	7a ₁	156.1	7a ₂	197.2
8a ₁	5.72 (ddd, 8.0, 10.0, 18.0)	8a ₂	5.57 (ddd, 7.5, 10.0, 17.5)	8a ₁	135.5	8a ₂	135.4
9a ₁	2.51 (ddd, 6.4, 6.0, 7.3) ^P	9a ₂	2.75 (ddd, 5.5, 5.7, 8.5) ^P	9a ₁	46.6	9a ₂	45.3
10a ₁	5.32 (d, 17.0) 5.25 (d, 10.5)	10a ₂	4.99 (d, 19.5) 4.96 (d, 11.5)	10a ₁	120.6	10a ₂	119.6
				11a ₁	168.2	11a ₂	168.2
1'a ₁	4.61 (d, 8.0)	1'a ₂	4.61 (d, 8.0)	1'a ₁	101.2	1'a ₂	101.2
2'a ₁	3.14~3.35	2'a ₂	3.14~3.35	2'a ₁	74.7	2'a ₂	74.6
3'a ₁	3.14~3.35	3'a ₂	3.14~3.35	3'a ₁	78.0	3'a ₂	78.0
4'a ₁	3.14~3.35	4'a ₂	3.14~3.35	4'a ₁	71.6	4'a ₂	71.5
5'a ₁	3.14~3.35	5'a ₂	3.14~3.35	5'a ₁	78.3	5'a ₂	78.3
6'a ₁	3.58~3.66 3.82~3.86	6'a ₂	3.58~3.66 3.82~3.86	6'a ₁	62.8	6'a ₂	62.8
1b ₁	5.12 (4.0) ^P	1b ₂	5.20 (d, 5.5)	1b ₁	97.9	1b ₂	97.7
3b ₁	7.38 (s)	3b ₂	7.39 (s)	3b ₁	153.2	3b ₂	152.7
4b ₁		4b ₂		4b ₁	112.6	4b ₂	111.1
5b ₁	3.06 (br q, 8.0)	5b ₂	2.96 (ddd, 7.5, 9.5, 10.0) ^P	5b ₁	33.6	5b ₂	33.0
6b ₁	1.71 (ddd, 5.0, 8.0, 13.5) 2.26 (br dd, 7.0, 14.5)	6b ₂	1.55 (ddd, 4.5, 8.5, 9.0) 2.08 (dd, 8.5, 14.0)	6b ₁	40.4	6b ₂	40.3
7b ₁	5.21 (d, 5.5)	7b ₂	5.13 (d, 6.0)	7b ₁	78.6	7b ₂	77.8
8b ₁	2.14 (m)	8b ₂	2.14 (m)	8b ₁	41.4	8b ₂	41.1
9b ₁	2.01 (ddd, 5.0, 7.0)	9b ₂	1.90 (ddd, 5.0, 7.0, 7.5)	9b ₁	47.1	9b ₂	46.9
10b ₁	1.03 (3H, d, 6.5)	10b ₂	0.95 (3H, d, 6.5)	10b ₁	14.3	10b ₂	14.2
				11b ₁	169.4	11b ₂	169.4
MeO	3.64 (s)	MeO	3.64 (s)	MeO	51.9	MeO	51.8
1'b ₁	4.61 (d, 8.0)	1'b ₂	4.61 (d, 8.0)	1'b ₁	99.6	1'b ₂	100.4
2'b ₁	3.14~3.35	2'b ₂	3.14~3.35	2'b ₁	74.7	2'b ₂	74.7
3'b ₁	3.14~3.35	3'b ₂	3.14~3.35	3'b ₁	78.2	3'b ₂	78.2
4'b ₁	3.14~3.35	4'b ₂	3.14~3.35	4'b ₁	71.6	4'b ₂	71.6
5'b ₁	3.14~3.35	5'b ₂	3.14~3.35	5'b ₁	78.4	5'b ₂	78.4
6'b ₁	3.58~3.66 3.82~3.86	6'b ₂	3.58~3.66 3.82~3.86	6'b ₁	62.8	6'b ₂	62.8

*DEPT, HMQC, and HMBC experiments resulted in the unambiguous assignments of all proton and carbon signals

*^P The coupling constants were read from the ^1H NMR spectra in pyridine-d₅.

Table 2. ^1H - and ^{13}C - NMR spectral data for compound **2** in CD_3OD (500 MHz for ^1H and 125 MHz for ^{13}C)

^1H		^1H		^{13}C		^{13}C	
1a ₁	5.34 (6.5)	1a ₂	5.46 (d, 5.5)	1a ₁	97.5	1a ₂	98.0
3a ₁	7.60 (s)	3a ₂	7.47(s)	3a ₁	154.9	3a ₂	154.0
4a ₁		4a ₂		4a ₁	110.4	4a ₂	110.0
5a ₁	2.93 (dd, 4.0)	5a ₂	4.06 (d, 6.0)	5a ₁	33.0	5a ₂	34.2
6a ₁	1.31 (2H, d, 6.5)	6a ₂		6a ₁	21.4	6a ₂	141.1
7a ₁	6.30 (t, 8.0)	7a ₂	10.00 (s)	7a ₁	150.8	7a ₂	192.1
8a ₁	5.37 (ddd, 6.5, 11.0, 17.5)	8a ₂	5.71 (ddd, 8.0, 10.5, 17.5)	8a ₁	135.4	8a ₂	135.2
9a ₁	2.67 (ddd, 4.6, 5.8, 6.1) ^P	9a ₂	2.67 (ddd, 5.5, 6.0, 7.5) ^P	9a ₁	45.8	9a ₂	45.2
10a ₁	5.02 (d, 11.0)	10a ₂	5.22 (d, 11.0)	10a ₁	120.5	10a ₂	120.1
	5.08 (d, 17.5)		5.27 (d, 17.5)	11a ₁	168.0	11a ₂	167.7
1'a ₁	4.66 (d, 8.0)	1'a ₂	4.63 (d, 8.0)	1'a ₁	100.3	1'a ₂	100.1
2'a ₁	3.11~3.33	2'a ₂	3.11~3.33	2'a ₁	74.6	2'a ₂	74.6
3'a ₁	3.11~3.33	3'a ₂	3.11~3.33	3'a ₁	78.0	3'a ₂	78.0
4'a ₁	3.11~3.33	4'a ₂	3.11~3.33	4'a ₁	71.5	4'a ₂	71.4
5'a ₁	3.11~3.33	5'a ₂	3.11~3.33	5'a ₁	78.4	5'a ₂	78.4
6'a ₁	3.60~3.64	6'a ₂	3.60~3.64	6'a ₁	62.8	6'a ₂	62.6
	3.80~3.86		3.80~3.86				
1b ₁	5.15 (d, 5.5)	1b ₂	5.24 (d, 5.0)	1b ₁	97.8	1b ₂	97.5
3b ₁	7.37 (s)	3b ₂	7.37 (s)	3b ₁	152.8	3b ₂	152.6
				4b ₁	113.1	4b ₂	113.1
5b ₁	3.04(dd, 8.0)	5b ₂	2.90 (ddd, 7.0, 8.0, 8.5)	5b ₁	33.0	5b ₂	32.6
6b ₁	1.59 (ddd, 5.0, 8.5, 14.0)	6b ₂	1.69~1.74 (m)	6b ₁	40.6	6b ₂	40.2
	2.16 (dd, 8.0, 14.0)		2.24 (dd, 8.5, 14.0)				
7b ₁	5.16~5.18 (d, 6.0) ^P	7b ₂	5.02~5.10 (d, 6.0) ^P	7b ₁	78.7	7b ₂	77.3
8b ₁	2.02 (m)	8b ₂	2.02 (m)	8b ₁	41.2	8b ₂	41.0
9b ₁	2.09 (ddd, 6.5, 7.0, 8.0)	9b ₂	1.91 (ddd, 5.5, 6.5, 8.5)	9b ₁	47.1	9b ₂	46.8
10b ₁	1.03 (3H, d, 6.5)	10b ₂	1.01 (3H, d, 6.5)	10b ₁	14.2	10b ₂	14.1
				11b ₁	169.4	11b ₂	169.4
MeO	3.25 (s)	MeO	3.25 (s)	MeO	51.9	MeO	51.9
1'b ₁	4.60 (d, 8.0)	1'b ₂	4.60 (d, 8.0)	1'b ₁	100.2	1'b ₂	100.2
2'b ₁	3.11~3.33	2'b ₂	3.11~3.33	2'b ₁	74.7	2'b ₂	74.7
3'b ₁	3.11~3.33	3'b ₂	3.11~3.33	3'b ₁	78.0	3'b ₂	78.2
4'b ₁	3.11~3.33	4'b ₂	3.11~3.33	4'b ₁	71.6	4'b ₂	71.6
5'b ₁	3.11~3.33	5'b ₂	3.11~3.33	5'b ₁	78.4	5'b ₂	78.4
6'b ₁	3.60~3.64	6'b ₂	3.60~3.64	6'b ₁	62.8	6'b ₂	62.8
	3.80~3.86		3.80~3.86				

*DEPT, HMQC, and HMBC experiments resulted in the unambiguous assignments of all proton and carbon signals

*^P The coupling constants were read from the ^1H NMR spectra in pyridine-d₅.

Table 3. The IC₅₀ (μg/mL) values of butanol parts

Extracts	A549	Bel7402	BGC-823	HCT-8	A2780
XD-B-1	7.250	>10	>10	>10	>10
XD-B-2	6.151	5.326	5.900	5.670	5.156
XD-B-3	6.324	5.628	5.841	5.586	6.049
XD-B-4	>10	>10	>10	>10	>10
XD-B-5	>10	>10	>10	>10	>10
XD-B-6	>10	>10	>10	>10	>10

The IR, MS, and NMR spectra of compound **1**

Date: Wed Sep 21 16:47:30 2005

Sample Name: xy - XD - 17 (KBr)

Scans: 64

检测单位: 国家药物及代谢产物分析研究中心

Resolution: 4.000

美国尼高力公司(Nicolet Co.)傅立叶变换红外光谱仪: IMPACT - 400

Max. 3078.3 cps

+Q1: 3.513 to 3.964 min from Sample 1 (TuneSampleID) of bxy-XD-17.wiff (Turbo Spray), subtracted (6.222 to 9.333min), Smoot...

Data by: XD-17
 Sample Name:
 Description:
 Ionization Mode: ESI+
 History: Determine m/z Peak Detect[Centroid:30,Area];Correct Base[5.0%];Average[MS[1] 1.812..2....
 Created by: Accutof
 Mass Calibration data: (Internal Mass Calibration Data)
 Operator: Accutof
 Acquired: 9/27/2005 5:30:25 PM
 Created: 9/27/2005 5:35:12 PM
 Charge number: 1
 Element: ^{12}C : 0 .. 100, ^1H : 0 .. 200, ^{23}Na : 1 .. 1, ^{16}O : 0 .. 40
 Tolerance: 5.00 (mmu)
 Unsaturation Number: -1.5 .. 30.0 (Fraction: Both)

Mass	Calc. Mass	Mass Difference (mmu)	Mass Difference (ppm)	^{12}C	^1H	^{23}Na	^{16}O	Unsaturation Number
1497.50381	1497.50586	-2.05	-1.37	66	90	1	37	21.9

INOVA-501 1H-NMR txy-XD-17 IN CD3OD 05.09.02

Archive directory: /export/home/vnmr1/vnmrsys/data

Sample directory:

File: 170101

File: 170101

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INOVA-501 1H-NMR XD-17 IN C5D5N 05.12.28

Archive directory: /export/home/vnmr1/vnmrsys/data

INDVA-501 1H-NMR XD-17 IN C5D5N 05.12.28
 Archive directory: /export/home/vnmr1/vnmrsys/data

1642.16
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 1628.43
 1623.66
 1618.91
 1608.92
 1601.46

1454.39
 1444.99
 1436.12
 1429.76
 1422.40
 1416.43

1329.23
 1322.05
 1313.08
 1304.96
 1297.49

INOVA-500 DEPT-NMR TXY-XD-17 IN CD3OD

INOVA-500 GCOSY-NMR TXY-XD-17 IN CD300

Solvent: CD300
Temp: 25.0 C / 298.1 K
INOVA-500 "NMR500"

Relax. delay 1.000 sec
Acq. time 0.220 sec
Width 4848.8 Hz
2D width 4848.8 Hz
4 repetitions

400 increments
OBSERVE H1, 500.0344850 MHz
DATA PROCESSING 0.005 sec
S4, sine bell 0.005 sec
F1, 0.0000000 sec
S4, sine bell 0.001 sec
F2, 0.0000000 sec
FT size 8192 x 8192
Total time 34 min, 21 sec

INOVA-500 HSQC-NMR TXY-XD-17 IN CD300

Solvent: CD300
Temp: 25.0 C / 298.1 K
User: L1177
INOVA-500 "NMR500"

Relax: delay 1.000 sec
Acq: time 0.220 sec
Wdth: 4048 Hz
2D Width 20817.1 Hz
48 repetitions

2 x 256 increments
OBSERVE H1, 500.0944850 MHz
DECOUPLE C13, 125.7593765 MHz

Power 45 dB
on during acquisition
off during delay
GARP-1 modulated

DATA PROCESSING
Gauss apodization 0.038 sec

F1 DATA PROCESSING
Gauss apodization 0.006 sec

FT size 2048 x 4096
Total time 8 hr, 51 min, 6 sec

INDVA-501 NOESY1D txy-XD-17 IN CD3OD 06.01.17
 Archive directory: /export/home/vmem1/vmemsys/data

INOVA-501 NOESY10 txy-XD-17 IN CD3OD 06.01.17
Archive directory: /export/home/vnmr1/vnmrsys/data

INOVA-501 NOESY1D txy-XB-17 IN CD3OD 06.01.17
 Archive directory: /export/home/vmar1/vmarsys/data

INOVA-501 NOESY1D txy-XD-17 IN CD3OD 06.01.17
Archive directory: /export/home/vnmr1/vnmrsys/data

The IR, MS, and NMR spectra of compound **2**

Date: Wed Sep 21 16:30:37 2005

Scans: 64

Resolution: 4.000

Sample Name: txy - XD - 14a (KBr)

检测单位: 国家药物及代谢产物分析研究中心

美国尼高力公司(Nicolet Co.)傅立叶变换红外光谱仪: IMPACT - 400

Data:xy-XD-14a
 Sample Name:
 Description:
 Ionization Mode:ESI+
 History:Determine m/z[Peak Detect[Centroid,30,Area];Correct Base[];Average(MS[1] 0.817..1
 Acquired:9/27/2005 5:19:26 PM
 Operator:Accutof
 Mass Calibration data:(Internal Mass Calibration Data)
 Created:9/27/2005 5:25:05 PM
 Created by:Accutof

Charge number:1
 Element:¹²C:0 .. 100, ¹H:0 .. 200, ²³Na:1 .. 1, ¹⁶O:0 .. 40
 Tolerance:5.00(mmu)
 Unsaturation Number:-1.5 .. 30.0 (Fraction:Both)

Mass	Calc. Mass	Mass Difference (mmu)	Mass Difference (ppm)	¹² C	¹ H	²³ Na	¹⁶ O	Unsaturation Number
1497.50208	1497.50586	-3.78	-2.53	66	90	1	37	21.5

Archive directory: /export/home/vnmr1/vnmrsys/data

INOVA-501 gHMBC txy-X0-14A in CD300 05.09.20
 Archive directory: /export/home/autogw/vnmrsys/data
 Sample directory: auto_04Sep2005
 File: CARBON
 Pulse Sequence: gHMBC
 Solvent: CD300
 Temp: 25.0 C / 238.1 K
 User: 1-14-87
 INOVA-500 "NMR501"
 Relax. delay 1.000 sec
 Acq. time 0.202 sec
 Spectroscopy
 2D Width 24502.3 Hz
 128 repetitions
 320 increments
 OBSERVE H1, 499.7470286 MHz
 DATA PROCESSING
 Processing time 0.0 sec
 F1 DATA PROCESSING
 Sine bell 0.005 sec
 FT size 2048 x 4096
 Total time 14 hr, 41 min, 19 sec

INDVA-501 1H-NMR txy-XD-14a IN CD3OD 06.01.17
 Archive directory: /export/home/vnmr1/vnmrsys/data

INOVA-501 NEDESYID txy-XD-14a IN CD300 06.01.17
 Archive directory: /export/home/vnmr1/vnmrsys/data