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Chemical constituents of Datura metel L.

Nguyen Thi Mai[1], Nguyen Thi Cuc[2], Tran Hong Quang[2*]

[1]*Hanoi University of Transport and Communications*

[2]*Institue of Marine Biochemistry, Vietnam Academy of Science and Technology (VAST)*

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Abstract

Chemical investigation of an acidic methanol extract of the whole plant of *Datura metel* resulted in the isolation of seven compounds, including pterodontriol B (**1**), disciferitriol (**2**), scopolamine (**3**), adenosine (**4**), thymidine (**5**), ilekudinoside C (**6**), and dioscoroside D (**7**). Their structures were elucidated by extensive spectroscopic methods, including 1D and 2D NMR and mass spectra and in comparison with reported data in the literature. Among the isolated compounds, pterodontriol B, disciferitriol, ilekudinoside C, and dioscoroside D were reported for the first time from the *Datura* genus.

Keywords. *Datura metel, Solanaceae*, sesquiterpene, triterpenoid saponin, steroidal saponin

1. INTRODUCTION

Datura metel L. is an annual herb that belongs to the Solanaceae family. It has tropical American origin and is widely cultivated in many tropical and temperate regions. In the Vietnamese traditional medicine, *D. metel* has been used for the treatment of coughs, bronchial asthma, and rheumatism [1]. Its leaves have been used as anesthetics in surgery, a fumigant in bronchial asthma, and anti-contractive agents in the stomach ulcers [1]. The flowers of *D. metel* have been used widely in the Chinese traditional medicine for the treatment of asthma, convulsions, pain, and rheumatism for centuries [2]. Previous studies of the pharmacological effects have shown that *D. metel* seeds exhibits a hypoglycemic activity in normal and alloxan-induced diabetic rats [3], the chloroform extract of *D. metel* displays an antifungal effect toward several pathogenic species of *Aspergillus* [4], and the seeds and fruit pulps of *D. metel* have a high antioxidant activity [5]. Chemical studies have demonstrated that the major chemical components of *D. metel* are withanolide-type steroids [6-12], which have been shown to suppress NO production in lipopolysaccharide (LPS)-stimulated RAW264.7 cells [11, 12], and exhibit cytotoxicity against HCT-116, A549, DLD-1, BGC-823, and K562 cancer cell lines [6, 7, 10]. In addition, the isolation of some megastigmane sesquiterpenes and amide alkaloids from *D. metel* was also reported [13, 14]. In the present study, we report the isolation and structural elucidation of seven compounds from the acidic methanol extract of the whole plants of *D. metel*.

2. MATERIAL AND METHODS

2.1. Plant material

The whole plants of *D. metel* were collected in Thai Binh province, Vietnam during May 2015, and identified by Dr. Bui Van Thanh, Institute of Ecology and Biological Resources. A voucher specimen (NCCT-CDM-5.2015) was deposited at the Herbarium of the Institute of Marine Biochemistry, VAST.

2.2. General experimental procedures

All NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C-NMR), chemical shifts are reported in ppm using TMS as an internal standard. ESIMS spectra were recorded on Agilent 1100. Column chromatography (CC) was performed on silica gel 230-400 mesh or RP-18 resins (150 μm, Fuji Silysia Chemical Ltd.). Compounds were visualized by spraying with aqueous 10% H_2SO_4 and heating for 5 minutes.

2.3. Extraction and isolation

The whole plant of *D. metel* was dried (5 kg), ground, and extracted with MeOH/acetic acid (pH = 5.0) under sonication at room temperature. After concentration under reduced pressure, the MeOH extract (300 g) was suspended in water and partitioned with $CHCl_3$ to give $CHCl_3$ and aqueous fractions. The aqueous fraction was alkalinized by adding NH_4OH until pH = 9.0, and then partitioned successively with CH_2Cl_2 and EtOAc to provide CH_2Cl_2, EtOAc, and aqueous fractions, respectively.

The CH_2Cl_2 and EtOAc fractions were combined and subjected to a reversed phase (RP) C_{18} column chromatography (CC), eluted with MeOH-H2O (10:17, v/v) to provide three subfractions (DME1-DME3). Subfraction DME1 was fractionated using a silica gel CC, being eluted with EtOAc-MeOH-H_2O (20:1:0.01, v/v/v) to give **3** (15 mg). The aqueous fraction was neutralized and subjected to fractionation through a Diaion HP-20 column, being eluted with a stepwise gradient of MeOH in water (25–100 %) to give four fractions (DMW1–DMW4). Fraction DMW2 was fractionated using reversed phase (RP) C_{18} column chromatography (CC), being eluted with MeOH-H_2O (1:3, v/v) to yield subfractions DMW21–DMW25. Subfraction DMW22 was then fractionated on a silica gel column, being eluting with CH_2Cl_2-MeOH-H_2O (5:1:0.05, v/v/v) to give **4** (6 mg) and **5** (8 mg). Fraction DMW4 was subjected to a RP C_{18} CC, being eluted with a stepwise gradient of MeOH-H_2O (1:2–4:1, v/v) to yield five subfractions (DMW41–DMW5). Fraction DMW42 was separated using a silica gel CC, being eluted with CH_2Cl_2-MeOH-H_2O (6:1:0.05, v/v/v) to provide four subfractions (DMW421–DMW424). Subfraction DMW421 was fractionated using a silica gel CC, being eluted with EtOAc-MeOH-H_2O (13:1:0.05, v/v/v) and further purified by a silica gel CC, being eluted with CH_2Cl_2-MeOH-H_2O (6:1:0.1, v/v/v) to give **2** (7 mg). Subfraction DMW424 was fractionated by a RP C_{18} CC, eluted with acetone-H_2O (1:3, v/v) and further purified by a silica gel CC, being eluted with CH_2Cl_2-MeOH-H_2O (6.5:1:0.05, v/v/v) to give **1** (7 mg). Subfraction DMW44 was fractionated using a silica gel CC, being eluted with EtOAc-MeOH-H_2O (2.5:1:0.1, v/v/v) to provide five subfractions (DMW441–DMW445). Subfraction DMW443 was fractionated by a RP C_{18} CC, being eluted with MeOH-H_2O (2:1, v/v) and further purified by a silica gel CC, being eluted with CH_2Cl_2-MeOH-H_2O (3:1:0.1, v/v/v) to yield **7** (12 mg), and **6** (10 mg).

Pterodontriol B (1): white, amorphous powder; $C_{15}H_{28}O_3$, M = 256; ESIMS: *m/z* 255 $[M–H]^–$; ^1H (CD_3OD, 500 MHz) and ^{13}C NMR data (CD_3OD, 125 MHz), see table 1.

Disciferitriol (2): white, amorphous powder; $C_{15}H_{28}O_3$, M = 256; ESIMS: *m/z* 255 $[M–H]^–$; ^1H (CD_3OD, 500 MHz) and ^{13}C NMR data (CD_3OD, 125 MHz), see table 1.

Scopolamine (3): white, amorphous powder; $C_{17}H_{21}NO_4$, M = 303; ESIMS: m/z 304 $[M+H]^+$; ^1H (CD_3OD, 500 MHz) and ^{13}C NMR data (CD_3OD, 125 MHz), see table 2.

Adenosine (4): white, amorphous powder; $C_{10}H_{13}N_5O_4$, M = 267; ESIMS: m/z 268 $[M+H]^+$; ^1H (CD_3OD, 500 MHz) and ^{13}C NMR data (CD_3OD, 125 MHz), see table 2.

Thymidine (5): white, amorphous powder; $C_{10}H_{14}N_2O_5$, M = 242; ESIMS: *m/z* 241 $[M–H]^–$; ^1H (CD_3OD, 500 MHz) and ^{13}C NMR data (CD_3OD, 125 MHz), see table 2.

Ilekudinoside C (6): white, amorphous powder; $C_{41}H_{66}O_{14}$, M = 782; ESIMS: *m/z* 805 $[M+Na]^+$; ^1H (CD_3OD, 500 MHz) and ^{13}C NMR data (CD_3OD, 125 MHz), see table 3.

Dioscoroside D (7): white, amorphous powder; $C_{51}H_{82}O_{22}$, M = 1046; HRESIMS: *m/z* 1081.4988 $[M+Cl]^–$ (calcd. for $C_{51}H_{82}ClO_{22}$, 1081.4986); ^1H (CD_3OD, 500 MHz) and ^{13}C NMR data (CD_3OD, 125 MHz), see table 3.

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white, amorphous powder. Its molecular formula was identified as $C_{15}H_{28}O_3$ by an ESIMS ion peak at *m/z* 255 $[M–H]^–$, along with the ^{13}C NMR data. The ^1H NMR of **1** showed the signals of four tertiary methyl groups at δ_H 1.26 (6H, s, H_3-12 and H_3-13), 1.11 (3H, s, H_3-14), and 0.93 (3H, s, H-15), and one oxymethine proton at δ_H 3.26 (1H, m, H-1) (Table 1). The ^{13}C NMR and DEPT spectra contained signals for 15 carbons, including three non-protonated carbons (two of which were oxygenated), three methines (of which one was oxygenated), five methylenes, and four methyl carbons. Comparison of the ^1H and ^{13}C NMR data of **1** with those of the reported *ent*-eudesmane sesquiterpene, pterodontriol B revealed that the structures of these compounds are similar [15]. The minor differences between the ^{13}C chemical shifts of these compounds observed at C-3, C-9, and C-11 might be due to the different solvents that these compounds were recorded in (**1**: in CD_3OD; pterodontriol B: in C_5D_5N). In the HMBC spectrum, the HMBC correlations from δ_H 1.26 (H_3-12 and H_3-13) to δ_C 43.1 (C-7) and 75.2 (C-11), from δ_H 1.11 (H_3-14) to δ_C 42.0 (C-3), 72.8 (C-4), and 48.4 (C-5), and from δ_H 0.93 (H_3-15) to δ_C 80.8 (C-1), 48.4 (C-5), 39.2 (C-9), and 39.8 (C-10) indicated that four methyl groups and three hydroxyl

groups are located at C-11, C-4, and C-10 positions (figure 2). Thus, compound **1** was determined to be pterodontriol B.

Compound **2** was isolated as a white, amorphous powder and its molecular formula was established as $C_{15}H_{28}O_3$ by the presence of an ion $[M–H]^-$ at *m/z* 255 in the ESIMS. The ^1H and ^{13}C NMR spectra of **2** were found nearly identical with those of **1**, except for significant difference of the chemical shift of C-7 (**2**: δ_H 1.33/ δ_C 50.7 vs **1**: δ_H 1.70/ δ_C 43.1) (table 1), suggesting that these compounds have different configuration at C-7. This was supported by a good agreement when comparing the ^1H and ^{13}C NMR data of **2** with those reported for the 7-epimer of **1**, disciferitriol (table 1) [15]. The different ^{13}C chemical shift between these compounds at C-11 could be explained by influence of the different solvents used (**2**: in CD_3OD; disciferitriol: in C_5D_5N). Therefore, compound **2** was identified as disciferitriol.

The molecular formula of compound **3** was determined to be $C_{17}H_{21}NO_4$ by the observation of an ion $[M+H]^+$ at *m/z* 304 in the ESIMS and ^{13}C NMR spectroscopic analysis. The ^1H NMR spectrum contained signals for five aromatic protons at δ_H 7.32 (3H) and 7.37 (2H) which were characteristic of a phenyl ring. The ^1H NMR spectrum further exhibited signals for oxygenated proton signals at δ_H

Table 1: ^1H and ^{13}C NMR data for compounds **1** and **2**

C	δ_C [#1]	**1**		δ_C [#2]	**2**	
		δ_C [a,b]	δ_H [a,c]		δ_C [a,b]	δ_H [a,c]
1	80.1	80.8	3.26 (m)	79.5	80.3	3.24 (m)
2	30.1	29.4	1.63*	30.4	29.3	1.66*
3	39.0	42.0	1.75 (m)	42.8	41.9	1.75 (m)
			1.53*			1.53 (m)
4	71.7	72.8		71.7	72.5	
5	48.2	48.4	1.64*	54.0	54.0	1.26*
6	21.8	21.7	2.03 (br d,	22.3	23.1	1.67 (m)
			13.5)/1.53*			1.29 (m)
7	42.8	43.1	1.70 (m)	50.6	50.7	1.33 (m)
8	21.9	21.6	1.80 (m)	23.1	22.6	1.95 (m)
			1.63 (m)			1.15 (m)
9	42.4	39.2	1.60*	42.4	42.0	1.95*
			1.53*			1.09*
10	39.6	39.8		40.1	40.1	
11	74.0	75.2		71.0	73.4	
12	29.8	28.7[d]	1.26 (s)[d]	27.9	26.8[d]	1.19 (s)[d]
13	30.4	29.5[d]	1.26 (s)[d]	28.0	27.4[d]	1.20 (s)[d]
14	23.1	22.0	1.11 (s)	23.4	22.5	1.11 (s)
15	14.4	14.1	0.93 (s)	14.0	13.7	0.88 (s)

[a]Recorded in CD_3OD, [b]125 MHz, [c]500MHz, [d] Signals are interchangeable; *Overlapped signal; [#1] δ_C of pterodontriol B [15] in C_5D_5N; [#2] δ_C of disciferitriol [15] in C_5D_5N

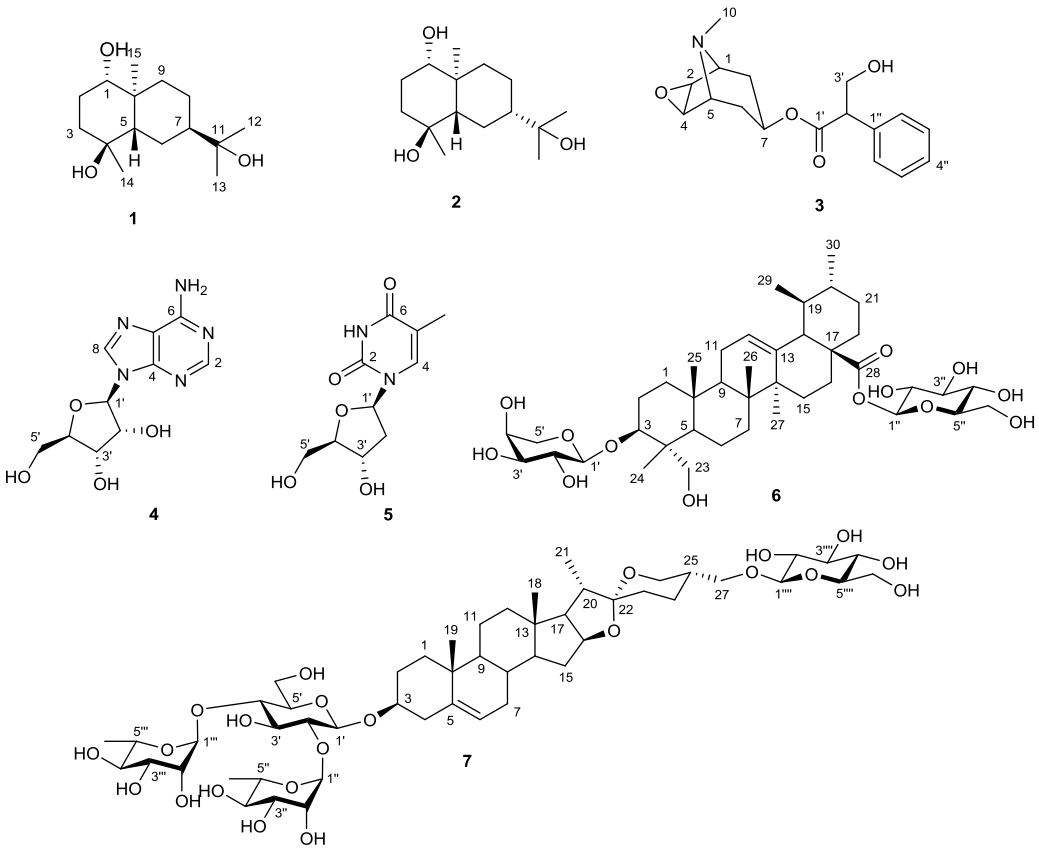

Figure 1: Chemical structures of compounds **1-7** from *D. metel*

Table 2: ^{1}H and ^{13}C NMR data for compounds **3-5**

C	δ_C [#1]	**3**		δ_C [#2]	**4**		δ_C [#3]	**5**	
		δ_C [a,b]	δ_H [a,c]		δ_C [d,b]	δ_H [d,c]		δ_C [a,b]	δ_H [a,c]
1	57.3	58.6	3.17 (m)						
2	55.8	56.4	3.57 (d, 3.5)	152.4	152.3	8.13 (s)	152.37	152.4	
4	55.4	56.1	3.03 (d, 3.5)	149.0	149.0		138.16	138.1	7.83 (d, 1.0)
5	59.2	58.7	3.28 (m)	119.3	119.3		111.51	111.5	
6	30.4	29.4	1.50 (d, 16.0) 2.13 (m)	156.2	156.1		166.42	166.5	
7	66.3	67.1	4.99 (t, 5.5)						
8	30.2	29.6	1.71 (d, 16.0) 2.22 (m)	139.9	139.8	8.34 (s)			
10	41.6	39.1	2.52 (s)						
1′	171.2	172.8		87.9	87.9	5.87 (d, 6.5)	86.25	86.2	6.30 (t, 7.0)
2′	53.8	55.9	3.79 (m)	73.4	73.4	4.59 (dd, 5.5, 6.5)	41.22	41.1	2.26 (m)
3′	63.4	64.4	3.77 (m) 4.15 (t, 11.0)	70.6	70.6	4.14 (dd, 3.0, 5.5)	72.21	72.2	4.42 (m)
4′				85.9	85.8	3.96 (m)	88.82	88.8	3.93 (m)
5′				61.6	61.6	3.53 (br d, 12.0) 3.67 (br d, 12.0)	62.84	62.8	3.82 (dd, 12.0, 3.0) 3.76 (dd, 12.0, 3.5)
1″	135.4	137.5							
2″, 6″	128.3	129.9	7.32*						
4″	127.6	128.8	7.32*						
3″, 5″	127.3	129.2	7.37*						
5-CH_3							12.44	12.4	1.90 (d, 1.0)

[a] Recorded in CD_3OD, [b]125 MHz, [c]500MHz, [d]in DMSO-d_6; * Overlapped signal; [#1] δ_C of scopolamine [16] in $CDCl_3$; [#2] δ_C of adenosine [17] in DMSO-d_6; [#3] δ_C of thymidine [18] in CD_3OD.

3.57 (d, J = 3.5 Hz, H-2), 3.03 (d, J = 3.5 Hz, H-4), 4.99 (t, J = 5.5 Hz, H-7), 3.77 (m, H-3′a), and 4.15 (t, J = 11.0 Hz, H-3′b), and two protons bearing nitrogens at δ_H 3.17 (m, H-1) and 3.28 (m, H-5), and the down-filed signal of a methyl group bearing nitrogen at δ_H 2.52 (H_3-10). Analysis of the ^{13}C NMR and HSQC indicated the presence of one carbonyl carbon at δ_C 172.8 (C-1′), five aromatic methines at δ_C 137.5 (C-1″), 129.9 (C-2″ and 6″), 128.8 (C-4″), and 129.2 (C-3″ and 5″), one epoxy group at δ_C 56.4 (C-2) and 56.1 (C-4), one oxymethine at δ_C 67.1 (C-7), one oxymethylene at δ_C 64.4 (C-3′), two methines bearing nitrogen at δ_C 58.6 (C-1) and 58.7 (C-5), two methylenes, and one methyl group.

The ^{1}H and ^{13}C NMR data of **3** (in CD_3OD) showed a similarity with those of scopolamine (in $CDCl_3$), suggesting that the structures of both compounds are identical (table 2) [16]. By the HMBC correlations observed between δ_H 2.52 (H_3-10) and δ_C 58.6 (C-1) and between δ_H 3.57 (H-2) and δ_H 3.03 (H-4) and δ_C 58.7 (C-5), the positions of the methyl and epoxy groups were assigned to C-10 and C-4/C-5, respectively (figure 2). The overall structure of **3** was subsequently assigned by the HMBC correlations between δ_H 3.79 (H-2′) and δ_C 137.5 (C-1″), 129.9 (C-2″ and C-6″), between δ_H 3.77 and 4.15 (H_2-3′) and δ_C 172.8 (C-1′), 55.9 (C-2′), and 137.5 (C-1″), and between δ_H 4.99 (H-7) and δ_C 172.8 (C-1′). Based on the above analysis, compound **3** was determined to be scopolamine.

The ESIMS of compound **4** exhibited an ion $[M+H]^+$ at *m/z* 268, corresponding with the molecular formula $C_{10}H_{13}N_5O_4$. The ^{1}H NMR spectrum showed signals of two down-field aromatic protons at δ_H 8.13 (s, H-2) and 8.34 (s, H-8), suggesting that these protons are connecting with nitrogen atoms. The ^{1}H NMR further displayed a signal for one anomeric proton at δ_H 5.87 (d, J = 6.5 Hz) revealing that **4** has one sugar moiety. The ^{13}C NMR contained 10 carbon signals, including two down-field aromatic methines at δ_C 152.3 (C-2) and 139.8 (C-8), three non-protonated carbons at δ_C 149.0, 119.3 (C-5), and 156.1, suggesting that **4**

possesses the purine nucleus. The five remaining carbon signals, including four oxymethines and one oxymethylene group were assigned to a β-ribofuranose by comparing with the data reported in the literature (table 2) [17]. Thus, compound **4** was identified as adenosine.

Table 3: ^{1}H and ^{13}C NMR data for compounds **6** and **7**

C	δ_C [#1]	**6**		δ_C [#2]	**7**		C	δ_C [#1]	**6**		δ_C [#2]	**7**	
		δ_C [a,b]	δ_H [a,c]		δ_C [a,b]	δ_H [a,c]			δ_C [a,b]	δ_H [a,c]		δ_C [a,b]	δHa,c
1	47.3	47.3	0.88* 2.05*	37.5	38.5	1.10*/1.90*	1′	106.6	106.2	4.31 (d, 7.5)	100.3	100.4	4.52 (d, 8.0)
2	66.9	68.0	3.81*	30.1	30.7	1.62*/1.94*	2′	73.1	72.9	3.60*	77.9	79.3	3.42 (dd 7.5, 9.5)
3	88.4	88.5	3.48 (d, 9.5)	78.1	79.3	3.61 (m)	3′	74.9	74.6	3.53*	77.8	77.9	3.61*
4	44.7	45.2		39.0	39.5	2.31 (t, 12.0)/2.48 (dd, 3.0, 13.0)	4′	69.7	70.0	3.84*	78.5	79.9	3.54 (t, 9.5)
5	47.7	47.7	1.22*	140.8	141.8		5′	67.8	67.8	3.65* 3.92 (d, 10.5)	76.9	76.5	3.34*
6	18.2	18.7	1.39*/1.49*	121.8	122.6	5.40 (d, 3.0)	6′				61.3	61.9	3.67* 3.82 (br d, 11.0)
7	33.2	33.6	1.33*/1.68*	32.3	33.1	1.58*/2.01*	1″	95.7	95.7	5.37 (d, 8.0)	102.0	102.3	5.22 (br s)
8	40.8	41.0		31.7	32.7	1.68*	2″	74.1	73.9	3.33*	72.5	72.1	3.95*
9	48.2	48.8	1.64*	50.3	51.6	0.99*	3″	78.9	78.2	3.42*	72.8	72.4	3.68*
10	37.8	38.5		37.1	38.0		4″	71.4	71.2	3.38*	74.1	73.9	3.43 (dd, 9.0, 9.5)
11	23.9	24.5	1.98*	21.1	21.9	1.56*	5″	79.2	78.5	3.36*	69.5	69.7	4.14*
12	126.2	127.0	5.27 (br s)	39.8	40.9	1.23*/1.79*	6″	62.5	62.5	3.82*/3.71*	18.6	17.8	1.27 (d, 6.5)
13	138.4	139.3		40.4	41.4		1″				102.9	102.9	4.86*
14	42.6	43.4		56.6	57.7	1.18*	2″				72.5	72.1	3.86*
15	28.7	29.2	1.11*/1.96*	32.2	32.7	1.31*/2.01*	3″				72.7	72.3	3.65*
16	24.7	25.2	1.78*/2.09*	81.1	82.2	4.42 (q, 7.5)	4″				73.9	73.7	3.43 (dd 9.0, 9.5)
17	48.4	48.4		62.8	63.7	1.78	5″				70.4	70.6	3.95*
18	53.4	54.1	2.27 (d, 12.0)	16.3	16.7	0.83 (s)	6″				18.5	17.9	1.28 (d, 6.0)
19	39.2	40.2	1.00*	19.4	19.8	1.07 (s)	1‴				105.0	104.7	4.22 (d, 7.5)
20	39.4	40.3	1.41*	42.0	42.9	1.93*	2‴				75.1	75.1	3.19*
21	30.8	30.6	1.31*	15.0	14.8	1.00 (d, 7.0)	3‴				78.6	78.0	3.36*
22	36.8	37.5	1.67*/ 1.79 (br d, 13.5)	109.5	110.8		4‴				71.6	71.6	3.29*
23	63.8	64.0	3.28 (d, 11.5)/3.71*	31.3	31.9	1.63* 1.73*	5‴				78.5	78.0	3.28*
24	14.7	14.4	0.76 (s)	23.9	24.2	1.57*/1.68*	6‴				62.8	62.7	3.69*/ 3.88*
25	17.4	17.8	1.07 (s)	36.7	37.1	1.91*							
26	17.6	17.9	0.86 (s)	63.6	64.4	3.52 (br d, 11.0) 3.76*							
27	23.8	24.0	1.15 (s)	72.0	72.8	3.33*/3.80*							
28	176.2	177.9											
29	17.8	17.6	0.92 (d, 6.5)										
30	21.3	21.5	1.30 (d, 6.5)										

[a] Recorded in CD_3OD, [b]125 MHz, [c]500MHz; *Overlapped signal; [#1] δ_C of ilekudinoside C [19] in C_5D_5N; [#2] δ_C of dioscoroside D in [20] C_5D_5N

The molecular formula of thymidine (**5**), $C_{10}H_{14}N_2O_5$ was deduced by its ESIMS ion at *m/z* 241 $[M–H]^-$ and ^1H and ^{13}C NMR spectra. The ^1H NMR spectrum showed signals for one aromatic proton at δ_H 7.83 (d, *J* = 1.0 Hz, H-4), one methyl group at δ_H 1.90 (d, *J* = 1.0 Hz, 5-C$\underline{H}_3$), and one anomeric proton at δ_H 6.30 (t, *J* = 7.0 Hz, H-1′). The ^{13}C NMR spectrum displayed 10 carbon signals, including two carbonyl carbons at δ_C 152.4 (C-2) and 166.5 (C-6), one aromatic methine at δ_C 138.1 (C-4), one non-protonated aromatic carbon at δ_C111.5 (C-5), and one methyl at δ_C 12.4 (5-$\underline{C}H_3$), suggesting the presence of a methyl-pyrimidinedione structural moiety. The remaining carbon signals, including three oxymethines, one oxymethylene, and one methylene carbons were assigned to a deoxy-*β*-D-ribofuranoside by comparing with the reported values (table 2) [18]. So the structure of thymidine (**5**) was established as shown in figure 1.

Ilekudinoside C (**6**) was isolated as a white, amorphous powder. Its molecular formula was $C_{53}H_{86}O_{21}$, as deduced by ESIMS at *m/z* 805 $[M+Na]^+$ and its ^{13}C NMR spectrum. The ^{13}C NMR spectrum exhibited 41 carcbon signals, of which 30 were assigned to a triterpenoid alglycone and 11 to a saccharide moiety. The ^1H NMR spectrum of **6** contained signals for six methyl groups at δ_H 0.76 (s, H_3-24), 1.07 (s, H_3-25), 0.86 (s, H_3-26), 1.15 (s, H_3-27), 0.92 (d, 6.5, H_3-29), and 1.30 (d, 6.5, H_3-30), a trisubstituted olefinic proton at δ_H 5.27 (br s), and two anomeric protons at δ_H 4.31 (d, 7.5, H-1′) and 5.37 (d, 8.0, H-1″). The signals at δ_C 127.0 and 139.3 in the ^{13}C NMR spectrum, assignable to C-12 and C-13, suggested the presence of a Δ^{12}-ursane-type triterpene. Signals at δ_C 88.5 (C-4) and 177.9 (C-28) in the ^{13}C NMR spectrum suggested that **6** is a bisdesmosidic ursane-type saponin. The sugar units were identified as one glucopyranose and one arabinopyranose based on comparing the ^{13}C NMR data of **6** with those reported previously in the literature [19]. The relatively large spin couplings of the anomeric protons (*J* > 7.5 Hz) were indicative of the *α*-arabinopyranose and *β*-glucopyranose. In the HMBC spectrum, the HMBC correlation from δ_H 3.48 (H-3) to δ_C 68.0 (C-2) suggested that a hydroxyl group is attached to C-2 position (Figure 2). The HMBC correlations between δ_H 4.31 (H-1′) and δ_C 88.5 (C-3) and between δ_H 5.37 (H-1″) and δ_C 177.9 (C-28) indicated that the arabinose and glucose were located at C-3 and C-28, respectively. Based on the data obtained and comparing with those of the reported compound (table 3) [19], the structure of ilekudinoside C (**6**) was elucidated as shown in figure 1.

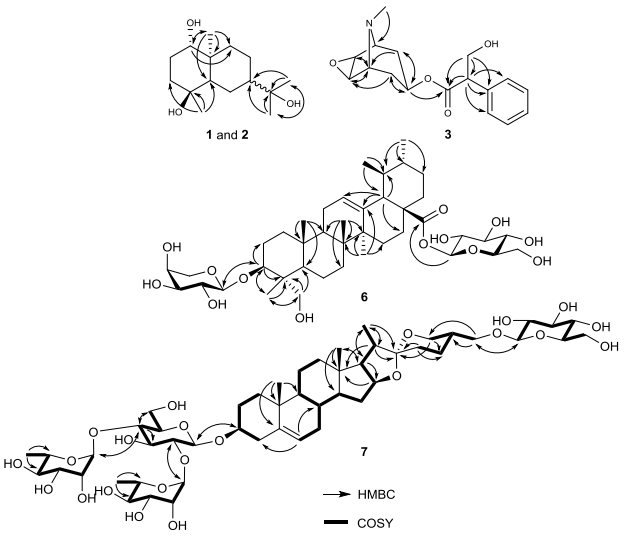

Figure 2: Selected HMBC correlations of compounds **1-3**, **6**, and **7** and COSY correlations of compound **7**

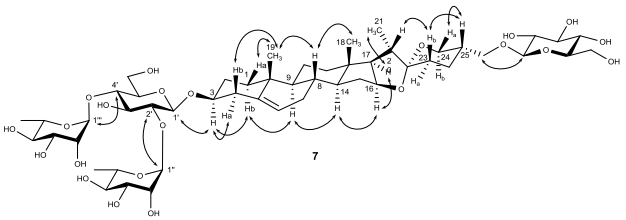

Figure 3: Selected ROESY correlations of compound **7**

Compound **7** had a molecular formula $C_{51}H_{82}O_{22}$, as deduced by the HRESIMS at m/z 1081.4988 $[M+Cl]^-$ (calcd. for $C_{51}H_{82}ClO_{22}$, 1081.4986) and the ^{13}C NMR data. The ^1H and ^{13}C NMR spectra, in combination with DEPT, HSQC, COSY, and HMBC spectra showed the presence of two tertiary methyls at δ_H 0.83 (s, H_3-18) and 1.07 (s, H_3-19), one secondary methyl group at δ_H 1.00 (d, *J* = 7.0 Hz, H_3-21), a trisubstituted olefinic proton at δ_H 5.40 (d, *J* = 3.0 Hz, H-6), and an acetalic carbon signal at δ_C 110.8 (C-22), implying that **7** possesses the $\Delta^{5,6}$-spirostane skeleton [21]. The ^1H NMR spectrum of **7** contained signals for four anomeric protons at δ_H 4.52 (d, *J* = 8.0 Hz, H-1′), 5.22 (br s, H-1″), 4.86 (H-1‴), and 4.22 (d, *J* = 7.5 Hz, H-1⁗), that showed HSQC correlations with anormeric carbons at δ_C 100.4 (C-1′), 102.3 (C-1″), 102.9 (C-1‴), and 104.7 (C-1⁗), respectively, indicating that **7** possesses four sugar units. Comparison of the ^{13}C NMR data of the sugar units with those reported previously suggested the presence of two glucopyranoses and two rhamnopyranoses. The relatively large coupling

constant (J > 7.5 Hz) of the anomeric proton of the glucose revealed the β-configuration, whereas the α-oriented anomeric form of the rhamnose was defined based on the chemical shift values of its C-3 and C-5 positions [22]. In the HMBC spectrum, the correlation between the anomeric proton of a glucose unit at δ_H 4.22 (H-1‴′) and δ_C 72.8 (C-27) indicated that this glucose was located at C-27 position of the aglycone (figure 2). The sugar sequence at C-3 of the aglycone was identified as α-L-rhamnopyranosyl-(1→4)[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside by the HMBC correlations between δ_H 4.86 (H-1‴) and δ_C 79.9 (Glc C-4), between δ_H 5.22 (H-1″) and δ_C 79.3 (Glc C-2), and between δ_H 4.52 (H-1′) and δ_C 79.3 (C-3). This assignment was also supported by the ROESY correlations between δ_H 4.86 (H-1‴) and δ_H 3.54 (H-4′), between δ_H 5.22 (H-1″) and δ_H 3.42 (H-2′), and between δ_H 4.52 (H-1′) and δ_H 3.61 (H-3) (figure 3). On the basis of the above analysis, along with comparison of the NMR data of **7** with those of the very recently reported spirostane-type saponin, the structure of compound **7** was established as shown in Figure 1, namely dioscoroside D [20].

In summary, our phytochemical study on the acidic methanol extract of *D. metel* resulted in the isolation and identification of seven compounds, including pterodontriol B (**1**), disciferitriol (**2**), scopolamine (**3**), adenosine (**4**), thymidine (**5**), ilekudinoside C (**6**), and dioscoroside D (**7**). Among the isolated compounds, pterodontriol B, disciferitriol, ilekudinoside C, and dioscoroside D were reported for the first time from the *Datura* genus.

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Corresponding author: **Tran Hong Quang**
Institute of Marine Biochemistry
Vietnam Academy of Science and Technology
No. 18, Hoang Quoc Viet, Cau Giay District, Hanoi
E-mail: quangth2004@yahoo.com.