Communications to the Editor

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Further Investigation of Antitumor Condurangoglycosides with C-18 Oxygenated Aglycone

Four antitumor active condurangoglycosides-B_0, -D_0, and 20-O-methyl- and 20-iso-O-methyl-condurangoglycoside-D_0 were isolated from Condurango Cortex. Their structures were established by chemical and spectral data. Their antitumor activities and toxicities are described.

Keywords——Condurango cortex; 18-oxygenated pregnane; condurangoglycosides-B_0, -D_0; 20-O-methyl- and 20-iso-O-methyl-condurangoglycoside-D_0; antitumor activity; ^{13}C-NMR

Two antitumor condurangoglycosides (CG)-A_0 (**1**) and -C_0 (**2**) from Condurango Cortex were reported in a previous communication.[1] We wish to describe here the structures and antitumor activities of four new glycosides, named condurangoglycosides-B_0 (**3**), -D_0 (**4**), 20-O-methyl-condurangoglycoside-D_0 (**5**) and 20-iso-O-methyl-condurangoglycoside-D_0 (**6**) in Chart 1 from the same source.

The physico–chemical data for these glycosides are given in Tables I, II, III, and IV. Their carbon nuclear magnetic resonance spectra help to determine their structures. Particularly, the chemical shifts due to the sugar moiety were basically identical with those of CGA_0 (**1**) or CGC_0 (**2**). Mild acid hydrolyses of these four glycosides afforded not only the same sugars but also aglycones, which consisted mainly of four components. In spite of the difficult separation of these aglycones because of their facile interconversion, one of the aglycones, condurangogenin B (**7**) $C_{32}H_{40}O_7$, was obtained. This compound has a diester moiety of acetic and cinnamic acids as indicated by its spectral data as shown in the tables. In addition

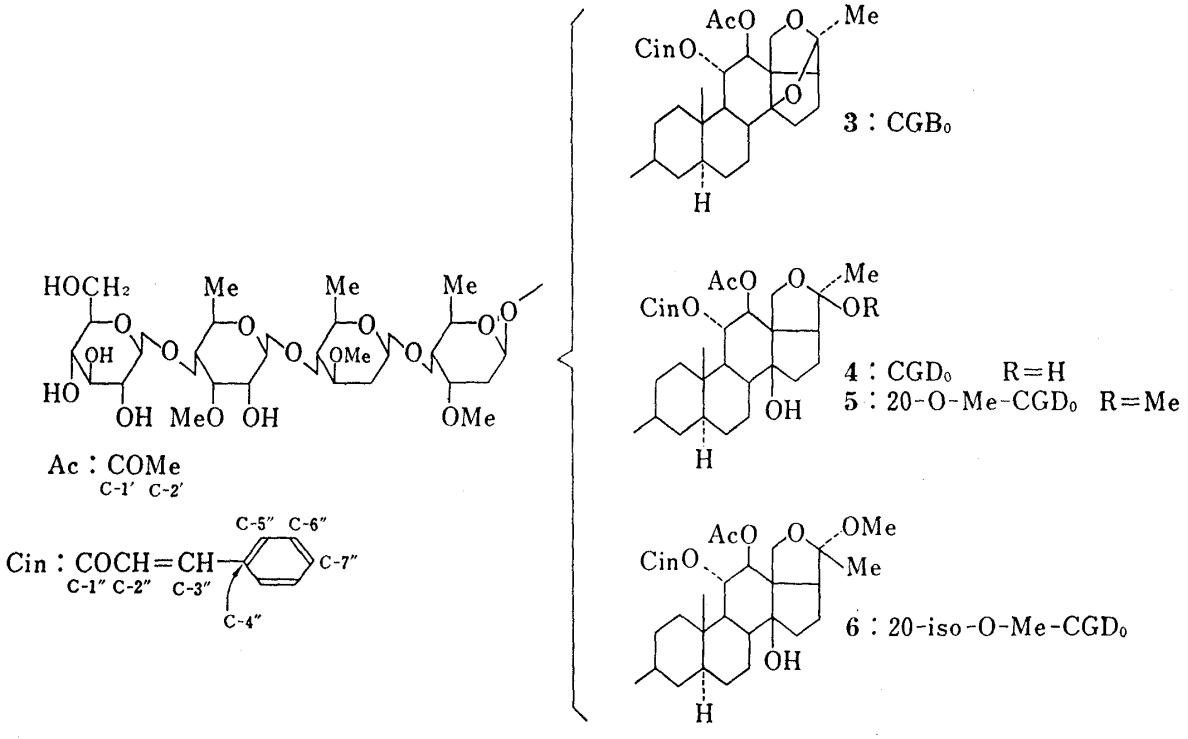


Chart 1

Table I. Physical Data of the Glycosides

	mp, $[\alpha]_D$, yield	*Anal.* and MS	UV λ_{max}^{EtOH} nm(log ε)	IR $\nu_{max}^{CHCl_3}$ cm^{-1}
3	170—180°C	$C_{59}H_{86}O_{22}\cdot 2H_2O$	217(4.51)	3350, 1735, 1710
	+11.5°	Calcd 59.88; 7.67	222(4.48)	1635, 1600, 1580
	(c=0.72, $CHCl_3$)	Found 59.72; 7.48	280(4.70)	1500, 1060—1100
	0.08%	m/z: 1149 $(M+1)^+$		1000, 900, 845
4	183—188°C	$C_{59}H_{88}O_{23}\cdot 4H_2O$	218(4.70)	3350, 1735, 1710
	+13.5°	Calcd 57.27; 7.82	221(4.15)	1635, 1600, 1500
	(c=0.99, $CHCl_3$)	Found 57.11; 7.36	280(4.36)	1160, 900, 860
	0.01%	m/z: 1169 $(M-H_2O+Na)^+$		
		1185 $(M-H_2O+K)^+$		
5	180—190°C	$C_{60}H_{90}O_{23}\cdot 4H_2O$	217(4.32)	3350, 1735, 1710
	−8.76°	Calcd 57.59; 7.89	222(4.28)	1635, 1600, 1580
	(c=0.72, $CHCl_3$)	Found 57.67; 7.42	289(4.49)	1500, 1160, 1100
	0.003%			960, 905, 880
6	168—173°C	$C_{60}H_{90}O_{23}\cdot 4H_2O$	218(4.31)	3400, 1735, 1710
	−19.0°	Calcd 57.59; 7.89	224(4.34)	1635, 1600, 1580
	(c=1.46, $CHCl_3$)	Found 57.97; 7.39	280(4.52)	1500, 1060—1100
	0.001%			950
7	115—123°C	m/z: 508 $(M-CH_2{=}CH_2)$	218(4.32)	3460, 1740, 1715
	+23.7°	416 $(M-2\times 60)$	223(4.23)	1635, 1380, 1260
	(c=0.47, $CHCl_3$)		281(4.54)	1230, 1160, 1040

Table II. Proton NMR Signals of the Glycosides

	δ ($CDCl_3$)
3	1.00 (3H, s, 19Me), 1.23 (3H, d, J=6 Hz), 1.27 (3H, d, J=6 Hz), 1.36 (3H, d, J=6 Hz), 1.40 (3H, s, 21Me), 1.92 (3H, s, Ac), 3.40, 3.46, 3.62 (each 3H, s), 4.15 (1H, br.d., J=8 Hz), 4.45 (2H, m), 4.83 (2H, br.d., J=8 Hz), 5.12 (1H, t, J=9 Hz), 5.21 (1H, d, J=6 Hz), 6.39 and 7.70 (2H, ABq, J=16 Hz)
4	1.00 (3H, s, 19Me), 1.2—1.35 (9H, m), 1.40 (3H, s, 21Me), 1.90 (3H, s, Ac), 3.38, 3.43, 3.49 (each 3H, s), 6.36 and 7.68 (2H, ABq J=16 Hz)
5	0.86 (3H, s, 19Me), 1.22 (3H, d, J=6 Hz), 1.24 (6H, d, J=6 Hz), 1.38 (3H, s, 21Me), 1.88 (3H, s, Ac), 3.27, 3.37, 3.43, 3.59 (each, 3H, s), 4.09 (2H, ABq, J=9 Hz, 18-CH_2-O), 6.44 and 7.73 (2H, ABq, J=16 Hz)
6	1.00 (3H, s, 19Me), 1.20—1.35 (9H, m), 1.40 (3H, s, 21Me), 1.90 (3H, s, Ac), 3.29, 3.35, 3.41, 3.56 (each, s, 3H), 6.35 and 7.66 (2H, ABq, J=16 Hz), 7.4 (5H, m)
7	1.02 (3H, s, 19Me), 1.40 (3H, s, 21Me), 1.91 (3H, s, Ac), 3.56 (1H, m, 3αH), 3.93 and 4.13 (2H, ABq, J=8 Hz, 18-CH_2-O), 5.09 (1H, d, J=10 Hz, 12αH), 5.21 (1H, t, J=10 Hz, 11βH), 6.37 and 7.67 (2H, ABq, J=16 Hz)

to only two steroidal methyl signals at δ 1.02 (C-19) and 1.40 (C-21) ppm, AB type doublets appeared at 3.93 and 4.13 ppm (J=8 Hz). This suggests the occurrence of oxygenation on the C-18 methyl group, which is supported by the ^{13}C NMR signals at 64.3 ppm (triplet). Two singlet signals at 90.3 and 113.2 ppm strongly suggest a ketal structure such as stapelogenin (**8**)[2] or holantosine (**9**).[3] The mass spectrum of **7** indicated that the cleavages of ethylene and acetic acid other than ester in the molecule occurred in a manner similar to that in **8**. Hydrogenation of **7** with Adam's catalyst as well as in the case of **8** afforded a perhydrocompound (**10**) which showed a new doublet methyl at 1.19 ppm (J=6.5 Hz) instead of the 1.40 singlet methyl in **7**. This signal was changed to a singlet by irradiation at 3.70 ppm multiplet corresponding to C-20 methine. The oxygenated methylene at C-18 at 3.63 and 4.05 ppm (AB doublets, J=9.5 Hz) and the vicinal protons at 4.84 (triplet, J=9.5 Hz, C-11βH) and 5.05 (doublet, J=9.5 Hz, C-12αH) ppm were also confirmed by decoupling experiments.

Table III. Carbon Chemical Shifts of Aglycone Moiety

Aglycone	7	3	4	5	6
C-1	38.2	37.3	37.4	38.0	37.5
C-2	32.8	30.4	30.4	30.4	30.1
C-3	71.0	76.0	76.2	76.2	76.2
C-4	39.8	35.6	35.4	35.5	35.5
C-5	45.4	44.7	44.6	44.6	45.0
C-6	28.6	28.3	28.5	28.5	28.5
C-7	29.2	28.9	29.3	29.3	29.2
C-8	35.3	37.8	42.1	41.7	41.5
C-9	51.5	51.3	49.7	49.4	51.5
C-10	38.2	38.3	37.8	37.8	37.5
C-11	72.7	72.0	72.0	71.9	71.6
C-12	72.9	72.4	76.7	76.9	76.5
C-13	57.6	57.6	65.2	64.7	62.2
C-14	90.3	90.1	81.8	81.0	83.3
C-15	18.0	17.9	26.5	19.6	25.9
C-16	30.0	29.7	38.4	38.5	35.8
C-17	51.7	51.2	58.3	59.6	59.7
C-18	64.4	64.1	64.4	65.3	67.2
C-19	12.7	12.4	12.1	12.1	12.4
C-20	113.2	112.8	103.8	105.8	109.4
C-21	16.5	16.4	20.8	20.5	17.4
$-OCH_3$				47.9	47.8
C-1′	170.1	170.0	170.1	170.1	169.3
C-2′	21.3	21.4	21.4	21.4	21.2
C-1″	166.0	166.1	166.8	166.9	166.0
C-2″	117.7	117.9	118.0	117.9	118.8
C-3″	146.1	146.1	146.2	146.4	145.3
C-4″	130.9	130.8	130.7	130.8	130.5
C-5″	129.2	129.3	129.1	129.2	129.2
C-6″	128.6	128.7	128.6	128.7	128.4
C-7″	134.6	134.7	134.7	134.5	135.0

Measured in pyridine-d_5.

Hypoiodate reaction of condurangogenin C-3-O-monoacetate (**11**) gave condurangogenin B acetate (**12**) accompanied by condurangogenin A acetate (**13**). This reaction confirmed the positions of both of the ester linkages in **3**, **4**, **5** and **6**.

These four glycosides, **3**, **4**, **5** and **6**, were converted into each other as follows. When CGB_0 (**3**) was treated with 0.005 N hydrochloric acid in methanol, it turned in to **6** after 20 h. In a diluted aqueous solution or 10^{-4} M acetic acid solution, **3**, **5** and **6** were converted in to **4**. Methylation of **4** with methyl iodide and silver oxide in DMF afforded **5** in 40% yield with 60% recovery of the starting material. Reflux of the suspension of **4** as well as **5** and **6** with $ZnCl_2$ in benzene caused their quantitative transformations to **3**. These facts indicate that the differences among these glycosides seemed to exist in the aglycone parts. Since the C-14, C-18 and C-20 carbons of **3** resonated at almost the same positions as those of **7**, CGB_0 (**3**) has the same ketal structure as **7**. The methylated glycoside 20-O-methyl-CGD_0 (**5**) and its epimer (**6**) had quartet signals at 47.9 and 47.8 ppm, respectively. Because the signals due to C-16 and C-21 carbons in **6** (35.8 and 17.4 ppm) resonated at a higher field than those of **5** (38.4 and 20.8 ppm), the C-16 and C-21 carbons of **6** should have a γ-gauche relation (Chart 3). Therefore, **6** has a C-20S configuration whereas **5** has a C-20R. The latter glycoside (**5**) and **4** seem to have the same stereochemistry at C-20 because the corresponding signals due to the C-16 and C-20 carbons between them have quite similar chemical shifts. Condurangoglycoside D_0 (**4**) should be hemiketal at C-20. The molecular formulae of **3** and **4** were confirmed by field desorption mass spectrometry. Since the methylated glycosides

Table IV. Carbon Chemical Shifts of Sugar Moiety

	3	4	5	6
Cy-1	96.1	96.0	96.1	96.2
Cy-2	37.6	37.4	37.6	37.4
Cy-3	77.9	77.9	77.9	77.8
Cy-4	82.7	82.6	82.6	82.7
Cy-5	68.9	68.8	68.8	68.9
Cy-6	18.2	18.1	18.1	18.1
3-OMe	58.8	58.8	58.8	58.8
Ole-1	101.7	101.6	101.7	101.7
Ole-2	37.8	37.5	37.8	37.6
Ole-3	79.3	79.4	79.3	79.3
Ole-4	83.3	83.3	83.3	83.3
Ole-5	72.0	72.0	72.0	72.0
Ole-6	18.7	18.6	18.7	18.7
3-OMe	57.2	57.2	57.2	57.2
Allo-1	101.8	101.8	101.8	101.8
Allo-2	72.6	72.6	72.6	72.6
Allo-3	83.4	83.3	83.3	83.3
Allo-4	83.0	82.9	83.0	83.0
Allo-5	69.5	69.4	69.5	69.4
Allo-6	18.9	18.8	18.8	18.9
3-OMe	61.6	61.5	61.6	61.6
Glu-1	106.4	106.3	106.4	106.4
Glu-2	75.4	75.4	75.4	75.4
Glu-3	78.2	78.0	78.2	78.2
Glu-4	72.0	72.0	72.0	72.0
Glu-5	78.2	78.2	78.3	78.3
Glu-6	63.1	63.2	63.1	63.2

Cy: cymarose, Ole: oleandrose, Allo: 6-deoxy-3-O-methyl-allose, Glu: glucose.
Measured in pyridine-d_5.

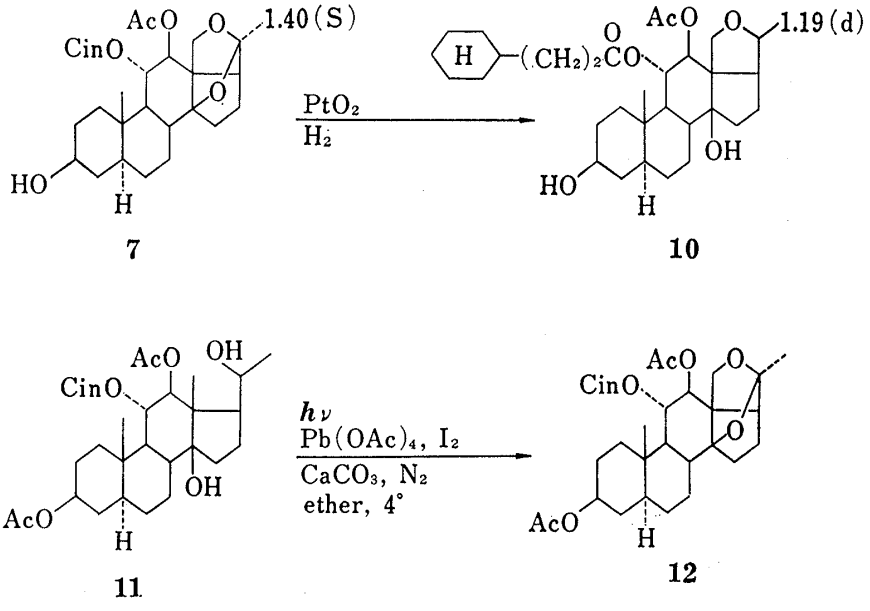
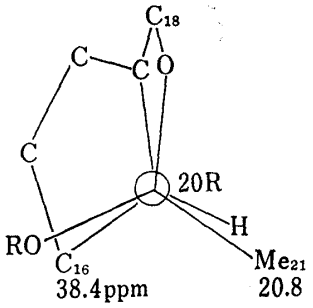


Chart 2

(5) and (6) were extracted even by solvents (*e.g.*, chloroform) other than methanol, they must have existed originally in the crude drug. However, their facile interconversion seemed to cause their proportion to vary with every commercial lot and separation process. Their

typical yields from the herb are shown in the Table I. The toxicities of **3**, **4**, **5**, and **6** and their antitumor activities, examined by slightly modified methods,[4] are shown in Table V.



4 : R=H
5 : R=Me

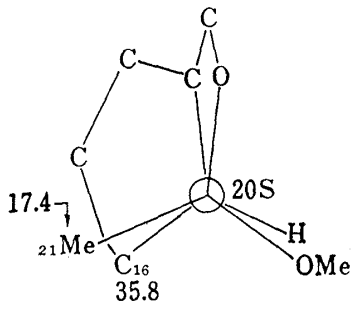


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Chart 3

TABLE V. Antitumor Activities and LD_{50} of **3**, **4**, **5**, and **6**

	Ehrlich carcinoma (T/C) (ddY mouse, dose: 16 mg/kg)	LD_{50} (mg/kg)
3	11.8	615
4	53.8	630
5	11.6	603
6	26.3	642

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References and Notes

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Reaction of 1-Naphthylhydroxylamine with Calf Thymus Deoxyribonucleic Acid. Isolation and Synthesis of N-(Guanin-C^8-yl)-1-naphthylamine

A possible activated form of 1-naphthylamine, N-hydroxy-1-naphthylamine, reacted with the C^8-position of guanine in deoxyribonucleic acid (DNA). Hydrolysis of the modified DNA with nuclease and acid gave N-(guanin-C^8-yl)-1-naphthylamine as a modified base. The C^8-position of guanine seems to be a common site of attack on DNA by activated muta-carcinogenic aromatic amines.

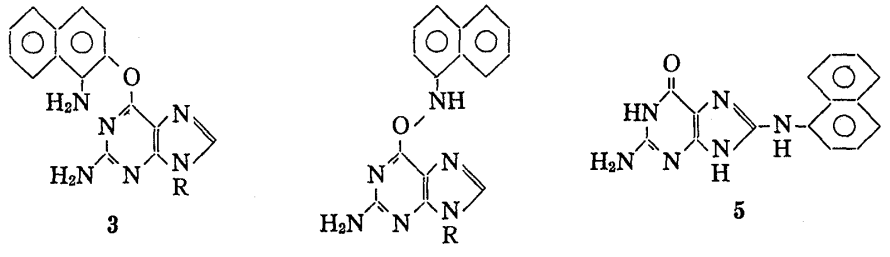
Keywords——1-naphthylamine; N-hydroxy-1-naphthylamine; aromatic hydroxylamine; modification of DNA; carcinogen; mutagen

In chemical carcinogenesis, metabolically activated carcinogens are thought to modify DNA. One important group of carcinogens such as 2-acetylaminofluorene (2-AAF), 4-dimethylaminoazobenzene (DAB), 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole (Trp-P-2) and 2-amino-6-methyldipyrido[1,2-*a*: 3′,2′-*d*]imidazole (Glu-P-1) is an aromatic amine and the metabolically activated forms of all these amino carcinogens are electrophilic hydroxylamine derivatives.

1-Naphthylamine (**1**, NA) is known to be a carcinogen of the urinary bladder,[1] and its N-hydroxy derivative, N-hydroxy-1-naphthylamine (**2**, N-OH-1-NA), is thought to be its metabolically activated form.[2] It has been reported that **2** binds to DNA and ribonucleic acid covalently at a slightly acidic pH, and the structures of the major modified nucleosides were proposed to be 2-(deoxyguanosin-O^6-yl)-1-naphthylamine (**3**) and N-(deoxyguanosin-O^6-yl)-1-naphthylamine (**4**).[3]

However, activated forms (electrophilic hydroxylamine derivatives) of many muta-carcinogenic aromatic amines attack the nucleophilic C^8-position of guanine in DNA: the major bases in DNA modified by 2-AAF and DAB are derivatives of the C^8-position of guanine.[4,5] Recently the activated forms of Trp-P-2 and Glu-P-1 were found to modify native DNA almost entirely at the C^8-position of its guanine.[6,7] This finding prompted us to examine modification of the C^8-position of guanine of DNA by N-OH-1-NA *in vitro*.

N-OH-1-NA was prepared by reduction of 1-nitronaphthalene by the method of



3 **4** **5**

Chart 1