STUDIES ON THE ACTIVE PRINCIPLES FROM ACONITUM FLAVUM HAND-MAZZ. THE STRUCTURES OF FIVE NEW DITERPENOID ALKALOIDS

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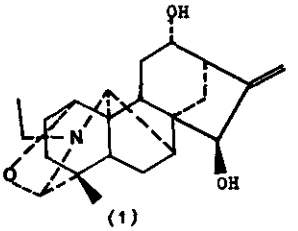
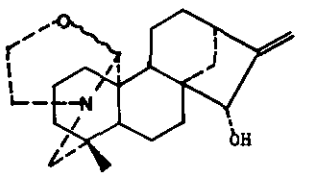
Abstract - Reinvestigation of Aconitum flavum Hand-Mazz resulted in the isolation of five new diterpenoid alkaloids, i.e., dehydronapelline (1), 12-acetyllucidusculine (2), 1-epi-napelline (3), 12-epi-napelline (4), and 1-demethylhypaconitine (9), along with napelline, lucidusculine, aconitine, 3-acetylaconitine, deoxyaconitine, flavaconitine, benzoylaconine, and neoline. The structures of these new compounds were determined based on spectral data and chemical transformations. The pharmacological tests showed that 12-acetyllucidusculine (2) has a strong effect of lowering of heart rate in Langendorff's guinea pig hearts.

In a previous paper[1] we reported the isolation of aconitine (10) and 3-acetylaconitine (11) from Aconitum flavum Hand-Mazz. Pharmacological tests[2] showed that 3-acetylaconitine (11) has strong analgesic and antiphlogistic effects. Now it is used as a drug for the treatment of periomethritis, rheumatic and rheumatoid arthrits, sciatica, lumbago and dorsalgia waist and joint sprain, etc. with non-narcotic addition after long duration of action. As the continuation of the search for pharmacological active components, we reinvestigated the constituents of the plant. From alcohol extract of the roots, we isolated five new diterpenoid alkaloids, named dehydronapelline (1), 12-acetyllucidusculine (2), 1-epi-napelline (3), 12-epi-napelline (4), and 1-demethylhypaconitine (9), along with six known compounds, napelline (5), lucidusculine (6), deoxyaconitine (12), flavaconitine (13), benzoylaconine (14), and neoline (16), in addition to the previous reported aconitine (10) and 3-acetylaconitine (11). The pharmacological tests revealed that C_{20} diterpenoid alkaloids, (1), (2), (5), and (6), all have effects of lowering of heart rate in Langendorff's gui-



(1)

dehydronapelline



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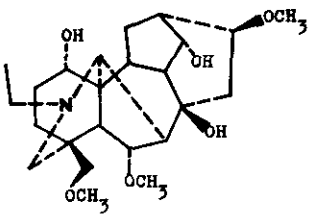

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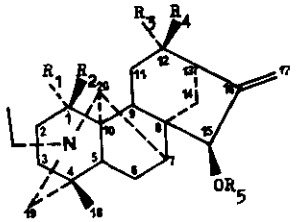


(2) R_1=OH, R_2=R_4=H, R_3=$OCOCH_3$, R_5=$COCH_3$.

12-acetyllucidusculine

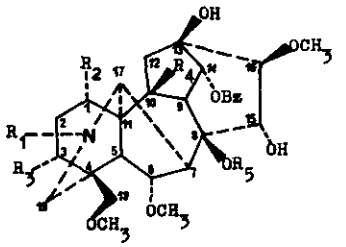
(3) R_1=R_4=R_5=H, R_2=R_3=OH. 1-epinapelline

(4) R_1=R_4=OH, R_2=R_3=R_5=H. 12-epinapelline

(5) R_1=R_3=OH, R_2=R_4=R_5=H.

(6) R_1=R_3=OH, R_2=R_4=H, R_5=$COCH_3$.

(7) R_1=OH, R_3, R_4=O, R_2=R_5=H.



(9) R_1=CH_3, R_2=OH, R_3=R_4=H, R_5=$COCH_3$.

(10) R_1=CH_2CH_3, R_2=OCH_3, R_3=OH, R_4=H, R_5=$COCH_3$.

(11) R_1=CH_2CH_3, R_2=OCH_3, R_3=$OCOCH_3$, R_4=H, R_5=$COCH_3$.

(12) R_1=CH_2CH_3, R_2=OCH_3, R_3=R_4=H, R_5=$COCH_3$.

(13) R_1=H, R_2=OH, R_3=H, R_4=OH, R_5=$COCH_3$.

(14) R_1=CH_2CH_3, R_2=OCH_3, R_3=OH, R_4=R_5=H.

(15) R_1=CH_3, R_2=OCH_3, R_3=R_4=H, R_5=$COCH_3$.

nea pig hearts. Among them, 12-acetyllucidusculine (2) is the strongest one and has no effect on the amplitude of cardiac contraction.

Dehydronapelline (1), $C_{22}H_{31}NO_3$ (M^+ 357.2274, calc. 357.2244), had mp 103.5-105°C, $[\alpha]_D^{16}$ +78.3° (C=0.48, EtOH). ^{1}H-nmr indicated the presence of an N-CH_2CH_3 (δ1.00, 3H, t, J=7.1 Hz: 2.61, 2.71, each 1H, m), a tertiary methyl (δ0.80, 3H, s), and two hydroxyls (δ1.69, 1H, d, J=4.0 Hz; 1.57, 1H, d, J=7.6 Hz, exchangable with D_2O). The ir absorptions at 1660, 890 cm^{-1} and the ^{1}H-nmr signal at δ5.16 (2H, brd, J=1.5 Hz, C=CH_2) suggested there was an exomethylene moiety. The molecular formula together with the spectral data suggested that this compound was a C_{20} diterpenoid alkaloid[5]. Decoupling correlative signals at δ1.14 (1H, dd, J=11.9, 4.3 Hz, C(14)-eH), 1.85 (1H, d, J=11.9 Hz, C(14)-aH), and 2.45 (1H, d, J=4.5 Hz, C(13)-H) indicated that this compound had napelline-type skeleton. Among the napelline-type compounds, the ring C exists in boat comformation because of chemical bond between C(7) and C(20). So the dihedral angle between C(13)-H and C(14)-aH or C(12)-βH is near 90°. There-

fore, C(13)-H and C(14)-2H appear to be characteristic of napelline-type compounds (see table I). The doublet of C(13)-H suggested that C(12)-αH was substituted, viz. there was a C(12)-αOH. When the ^{1}H-nmr signal at δ4.21 (1H, dt, J=7.6, 2.3 Hz) was irradiated, both signals at δ5.16 (2H, brd, J=1.5 Hz, C=CH_2) and 1.57 (1H, d, J=7.6 Hz, OH) were changed to siglet, suggesting the presence of C(15)-OH. The β-configuration of C(15)-OH (δ77.5) was established by observing the chemical shift of C(15) in napelline (5) (δ77.8, with β-OH)[3] and veatchine (8) (δ82.8, with α-OH)[6]. The mass spectrum exhibited the peaks at m/z 301 ($M^+-C_3H_4O$, 46%), 286 ($M^+-C_3H_4O-CH_3$, 29%). The loss of molecule of acrolein in the ms[5], the ir strong absorption at 1090 cm^{-1}, and the ^{1}H-nmr signals at δ3.68 (1H, s, C(19)-H) and 4.03 (1H, d, J=5.3 Hz, C(1)-βH) indicated the presence of C(1)-O-C(19) ether linkage in the new base[4]. Comparing the ^{13}C-nmr data with those of napelline (5) (see table II), the appearance of a new CH signal at δ93.1, the disappearance of a CH_2 signal at δ57.7, the upfield shift of C(1), the C(4) occurring downfield as the result of β-effect and the C(3), C(5), and C(18) occurring upfield as the result of γ-effect provided the evidences for the presence of C(1)-O-C(19) ether linkage. Based on these spectral analyses, this compound was assigned to be dehydronapelline (1). The oxidation of napelline with potassium permanganate afforded dehydronapelline[7] which was identical with the natural product. The reduction of this new compound with sodium borohyride produced a single product which was identical with napelline[8]. Therefore, the structure (1) was established.

12-Acetyllucidusculine (2), $C_{26}H_{37}NO_5$ (M^+ 443.2637, calc. 443.2603), mp 132-134°C, $[\alpha]_D^{26}$ -94.1° (C=0.46, $CHCl_3$), showed the character of napelline-type compound (see table I). The infrared spectrum exhibited absorptions at 3430 (hydroxyl), 1738, 1232, 1030 (ester), 1660 and 887 cm^{-1} (exomethylene). The ^{1}H-nmr spectrum was very similar to that of lucidusculine (6) except the appearance of an acetoxy (δ2.01, 3H, s) and 0.94 ppm downfield shift of C(12)-βH. Thus, this compound was assigned to be 12-acetyllucidusculine (2). Partial acetylation of lucidusculine (6) with acetic acid under the presence of trifluoroacetic acid at 80°C for 5 h. afforded 12-acetyllucidusculine (2)[3] (see fig. I). This fact confirmed the structure (2).

1-Epi-napelline (3) had mp 87-89°C, $[\alpha]_D^{22}$ -11.7°(C=1.19, CH_3OH) and gave an analysis corresponding to $C_{22}H_{33}NO_3$ (M^+ 359.2466, calc. 359.2460) by high resolution ms. The infrared spectrum showed absorptions at 3400 (hydroxyl), 1660 and 890 cm^{-1} (exomethylene). The mass spectrum of this base was in accord with that of napelline (5). Every carbon chemical shift was very similar to corresponding value of napelline (5)

Table I. The ^{1}H-nmr Data (400 MHz, δ, $CDCl_3$) for the Napelline Group Bases

Proron	5	6	7[a]	1	2	3[b]	4
C(18)-3H	0.80, s	0.78, s	0.70, s	0.80, s	0.74, s	0.80, s	0.79, s
N-$CH_2\underline{CH_3}$	1.16, t,	1.13, t,	1.03, t,	1.00, t,	1.04, t,	1.13, t,	1.18, t,
	J=6.8 Hz	J=6.6 Hz	J=7.2 Hz	J=6.8 Hz	J=6.8 Hz	J=7.1 Hz	J=7.1 Hz
C(14)-eH	1.07, dd,	1.18, dd,	1.45, dd,	1.14, dd,	1.17, dd,	1.00, dd,	1.09, dd,
	J=12.0, 4.2Hz	J=12.3, 4.3Hz	J=8.9, 4.0 Hz	J=11.9, 4.3Hz	J=12.4, 3.8Hz	J=12.0, 4.0Hz	J=12.1, 4.0Hz
C(14)-aH	1.94, d,	2.02, d,	2.21, d,	1.85, d,	1.98, d,	1.98, d,	1.76, d,
	J=12.1 Hz	J=12.1 Hz	J=8.9 Hz	J=11.9 Hz	J=12.8 Hz	J=12.0 Hz	J=12.2 Hz
C(13)-H	2.48, d,	2.48, d,	3.23, d,	2.45, d,	2.47, d,	2.38, d,	2.80, dd,
	J=4.1 Hz	J=4.0 Hz	J=3.9 Hz	J=4.5 Hz	J=3.4 Hz	J=3.7 Hz	J=8.8, 3.7 Hz
C(20)-H	3.53, brs	3.51, brs	3.71, brs	2.80, brs	3.37, brs	3.45, brs	3.52, brs
C(1)-H	3.91, dd,	3.92, dd,	3.97, dd,	4.03, d,	3.89, brt,	3.89, dd,	3.87, dd,
	J=8.6, 6.6 Hz	J=8.5, 6.8 Hz	J=10.4, 6.5Hz	J=5.3 Hz	J=7.1 Hz	J=9.9, 6.3 Hz	J=8.6, 6.7 Hz
C(12)-H	3.55, dd,	3.64, dd,		3.65, m	4.58, t,	3.52, dd,	4.18, dd,
	J=9.6, 6.7 Hz	J=9.3, 6.6 Hz			J=8.7 Hz	J=9.5, 7.0 Hz	J=8.8, 4.8 Hz
C(15)-H	4.20, dt,	5.52, t,	4.68, brs	4.21, dt,	5.49, brs	4.15, brs	4.21, brs
	J=7.8, 2.3 Hz	J=2.3 Hz		J=7.6, 2.3 Hz			
C(17)-2H	5.15, m	4.92, m	5.49, brs	5.16, brd,	4.97, brs	5.12, brs	5.12, brs
	5.18, m	5.13, m	5.54, brs	J=1.5 Hz	5.23, brs	5.15, brs	5.32, brs
C(19)-2H	2.32, 2.58,	2.26, 2.60,	2.19, 2.51,	3.68, 1H, s	2.20, 2.43,	2.09, 2.37,	2.30, 2.71,
	ABq, J=11.6Hz	ABq, J=11.1Hz	ABq, J=12.1Hz		ABq, J=11.3Hz	ABq, J=11.2Hz	ABq, J=11.8Hz
C(12)-$OCOCH_3$					2.01, s		
C(15)-$OCOCH_3$		2.11, s			2.09, s		

a) Spectrum was taken in pyridine-d_5.

b) Spectrum was taken in methanol-d_4.

Table II. The ^{13}C-nmr Data (100 MHz, δ, CD_3OD) for the Napelline Group Bases

Carbon	5	4	3	1[c]
1	70.5	69.5	77.0	67.9
2	31.9	31.5	31.2	29.9
3	32.4	32.4	32.4	24.4
4	34.7	35.2	35.3	37.8
5	49.4	51.6	49.1	32.5
6	23.6	24.3	24.1	24.0
7	45.0	45.1	45.8	46.7
8	50.3	51.6	51.0	50.4
9	38.2	39.6	38.1	45.9
10	53.5	53.8	54.0	51.9
11	29.4	33.6	29.7	27.8
12	76.2	71.8	76.7	76.4
13	49.9	45.7	50.6	48.8
14	38.4	38.8	38.5	30.4
15	77.8	78.1	78.4	77.5
16	160.8	154.8	160.0	157.7
17	107.4	112.2	108.4	109.5
18	26.4	26.7	26.8	19.0
19	57.7	58.9	59.0	93.1
20	66.2	67.3	67.0	66.0
N-CH_2	51.6	52.1	52.1	48.4
CH_3	13.3	13.7	13.7	14.3

c) Spectrum was taken in deuterochloroform.

Table III. The ^{13}C-nmr Data (100 MHz, δ, $CDCl_3$) for 1-demethylhypaconitine base

Carbon	9	15	16	Carbon	9	15	16
1	71.9	85.0	72.3	C=O	166.0	166.1	
2	29.4	26.4	29.5	1	129.5	129.9	
3	29.9	34.9	29.9	2	129.5	129.6	
4	38.1	39.3	38.2	3	128.6	128.6	
5	43.3	48.2	44.9	4	133.3	133.2	
6	83.5	83.1	83.3	5	128.6	128.6	
7	43.3	44.5	52.3	6	129.5	129.6	
8	91.7	91.9	74.3	1'		56.5	
9	42.2	43.8	48.3	6'	58.0	57.9	57.8
10	39.4	41.1	40.7	16'	61.4	60.9	56.3
11	49.2	49.9	49.6	18'	59.1	59.0	59.1
12	36.2	36.3	29.8				
13	73.9	74.1	44.3				
14	79.1	78.8	75.9				
15	78.7	78.8	42.7				
16	89.7	90.1	82.3				
17	64.4	62.1	63.6				
18	79.6	80.1	80.3				
19	58.5	56.0	57.2				
N-CH_3	42.7	42.6	NCH_2 48.2				
C=O	172.4	172.3	CH_3 13.0				
CH_3	21.3	21.4					

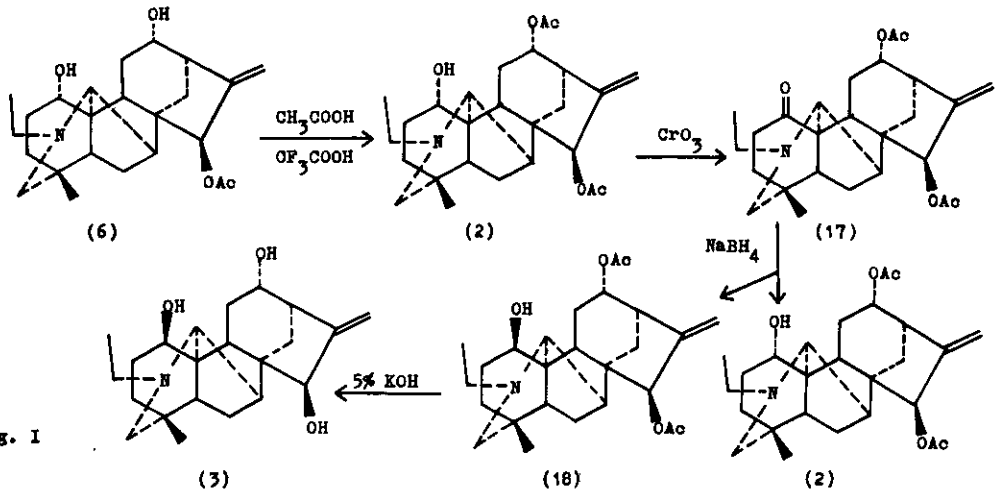


Fig. I

except C(1) (see table II). The 6.5 ppm downfield shift of C(1) suggested that the configuration of C(1)-OH was different from that of napelline (5), i.e., a β-orientation. Hence, this compound was deduced to be 1-epi-napelline (3). By oxidation with chromic anhydride 12-acetyllucidusculine (2) yielded 1-keto-12-acetyllucidusculine (17) (see fig. I) which, upon reduction with sodium borohydride, produced two products, 1-epi-12-acetyllucidusculine (18) and 12-acetyllucidusculine (2). The hydrolysis of the former one (18) afforded 1-epi-napelline (3) which was identical with the natural product and therefore, the structure (3) was established.

12-Epi-napelline (4), $C_{22}H_{33}NO_3$ (M^+ 359.2470, calc. 359.2460), mp 72-73.5°C, $[\alpha]_D^{21}$ -40.2°(C=1.03, $CHCl_3$), showed the character of napelline-type alkaloid (see table I). The double doublet of C(13)-H implied the presence of C(12)-αH. Irradiation at δ4.18 (1H, dd, J=8.8, 4.8 Hz) collapsed the signal of C(13)-H to doublet. This fact indicated that δ4.18 (1H, dd, J=8.8, 4.8 Hz) should be the signal of C(12)-αH and the C(12)-βH must be substituted by a hydroxyl group. The mass spectrum of this compound was in consistent with that of napelline (5). Comparison of the ^{13}C-nmr data with those of napelline (see table II) revealed that all of carbon chemical shifts were similar except those of ring C whose difference was respondent to the configurational change of the hydroxyl group at C(12). Therefore, 12-epi-napelline (4) was deduced to this new base. By reduction with lithium aluminium hydride, songorine (7) afforded two products, napelline (5) and 12-epi-napelline (4). The latter one was identical with the natural product, which confirmed the structure (4).

1-Demethylhypaconitine (9), $C_{32}H_{43}NO_{10}$ (M^+ 601.2839, calc. 601.2793), had mp 180-182°C, $[\alpha]_D^{26}$ +19.9°(C=0.38, $CHCl_3$). The infrared spectrum showed absorptions at 3500

(hydroxyl), 1725, 1290, 1150 (ester), 1605, 1589, 1490, and 713 cm^{-1} (aromatic ring). The ^{1}H-nmr spectrum in deuteriochloroform exhibited signals at δ1.39 (3H, s, C(8)-OCOCH_3), 2.38 (3H, s, N-CH_3), 3.15, 3.29, 3.75 (each 3H, s, OCH_3), 7.44-8.02 (5H, m, aromatic protons), 4.87 (1H, d, J=4.9 Hz, C(14)-βH), 3.18, 3.53 (each 1H, ABq, J=8.1 Hz, C(18)-2H), 3.97 (1H, d, J=6.4 Hz, C(6)-βH), 3.66 (1H, m, C(1)-βH), 3.40 (1H, d, J=5.2 Hz, C(16)-αH), 4.47 (1H, dd, J=5.4, 2.6 Hz, C(15)-βH), and 4.40 (1H, d, J=2.6 Hz, C(15)-OH). The ^{13}C-nmr spectrum of 1-demethylhypaconitine showed twenty-eight signals for thirty-two carbon atoms in the molecule. The pattern of carbon chemical shifts was similar to that of hypaconitine (15) except ring A (see table III). The appearance of a new CH signal at δ71.9, disappearance of C(1) signal at δ85.0, downfield shift of C(2) and upfield shifts of C(3), C(5) suggested that a secondary hydroxyl instead of a methoxy group must be present at C(1). The general carbon chemical shift for C(1) with an α-OH in aconitine-type alkaloids is δ72.0-73.0 except where a C(10)-OH or a C(2)-C(3) double bond is present[10]. The value for C(1) in delphirine (1-epi-neoline) with an β-OH is 69.0[10]. Since the shift for C(1) in this compound is 71.9, the C(1)-OH is assigned an α-orientation. Therefore, the structure (9) was assigned to 1-demethylhypaconitine.

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