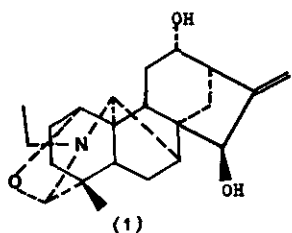


STUDIES ON THE ACTIVE PRINCIPLES FROM ACONITUM FLAVUM HAND-MAZZ.  
THE STRUCTURES OF FIVE NEW DITERPENOID ALKALOIDS

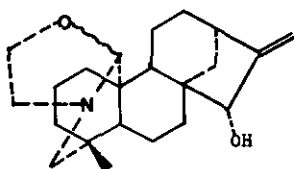
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Abstract - Reinvestigation of Aconitum flavum Hand-Mazz resulted in the isolation of five new diterpenoid alkaloids, i.e., dehydronapelline (1), 12-acetylucidusculine (2), 1-epi-napelline (3), 12-epi-napelline (4), and 1-demethylhyaconitine (9), along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

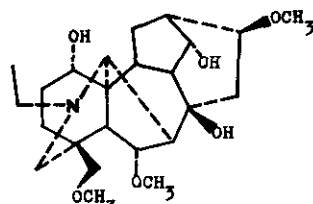
In a previous paper<sup>1</sup> we reported the isolation of aconitine (10) and 3-acetylaconitine (11) from Aconitum flavum Hand-Mazz. Pharmacological tests<sup>2</sup> showed that 3-acetylaconitine (11) has strong analgesic and antiphlogistic effects. Now it is used as a drug for the treatment of periostitis, rheumatic and rheumatoid arthritis, 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-acetylucidusculine (2), 1-epi-napelline (3), 12-epi-napelline (4), and 1-demethylhyaconitine (9), along with six known compounds, napelline (5), lucidusculine (6), deoxyaconitine (12), flavaconitine (13), benzoyleaconine (14), and neoline (16), in addition to the previous reported aconitine (10) and 3-acetylaconitine (11). The pharmacological tests revealed that C<sub>20</sub> diterpenoid alkaloids, (1), (2), (5), and (6), all have effects of lowering of heart rate in Langendorff's gui-



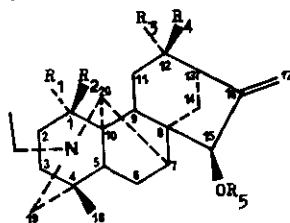
(1)  
dehydronapelline



(8)

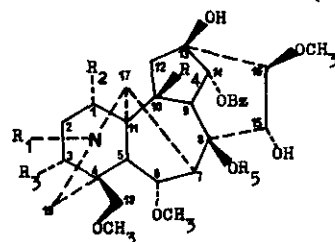


(16)



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

12-acetyllicidusculine



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

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

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

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

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

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

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

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

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

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

(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-acetyllicidusculine (2) is the strongest one and has no effect on the amplitude of cardiac contraction.

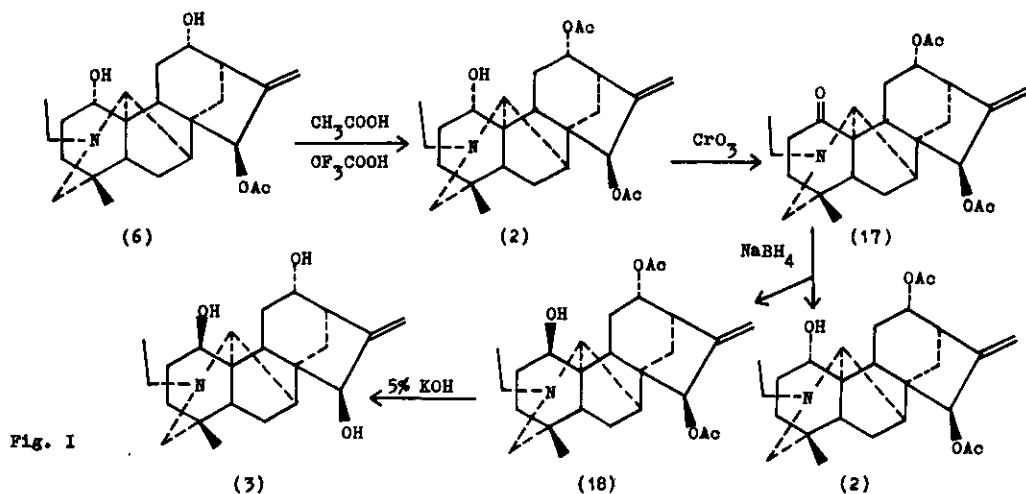
Dehydronapelline (1),  $C_{22}H_{31}NO_3$  ( $M^+$  357.2274, calc. 357.2244), had mp 103.5–105°C,  $[\alpha]_D^{16} +78.3^\circ$  ( $C=0.48$ , EtOH).  $^1H$ -nmr indicated the presence of an  $N-CH_2CH_3$  ( $\delta$ 1.00, 3H, t,  $J=7.1$  Hz; 2.61, 2.71, each 1H, m), a tertiary methyl ( $\delta$ 0.80, 3H, s), and two hydroxyls ( $\delta$ 1.69, 1H, d,  $J=4.0$  Hz; 1.57, 1H, d,  $J=7.6$  Hz, exchangeable with  $D_2O$ ). The ir absorptions at 1660, 890  $cm^{-1}$  and the  $^1H$ -nmr signal at  $\delta$ 5.16 (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<sup>5</sup>. Decoupling correlative signals at  $\delta$ 1.14 (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 conformation 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)- $\beta$ H 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)-OH was substituted, viz. there was a C(12)-OH. When the  $^1\text{H}$ -nmr signal at  $\delta$ 4.21 (1H, dt,  $J=7.6, 2.3$  Hz) was irradiated, both signals at  $\delta$ 5.16 (2H, brd,  $J=1.5$  Hz,  $\text{C}=\text{CH}_2$ ) and 1.57 (1H, d,  $J=7.6$  Hz, OH) were changed to siglet, suggesting the presence of C(15)-OH. The  $\beta$ -configuration of C(15)-OH ( $\delta$ 77.5) was established by observing the chemical shift of C(15) in napelline (5) ( $\delta$ 77.8, with  $\beta$ -OH)<sup>3</sup> and veatchine (8) ( $\delta$ 82.8, with  $\alpha$ -OH)<sup>6</sup>. The mass spectrum exhibited the peaks at  $m/z$  301 ( $\text{M}^+-\text{C}_3\text{H}_4\text{O}$ , 46%), 286 ( $\text{M}^+-\text{C}_3\text{H}_4\text{O}-\text{CH}_3$ , 29%). The loss of molecule of acrolein in the  $\text{ms}^5$ , the ir strong absorption at  $1090\text{ cm}^{-1}$ , and the  $^1\text{H}$ -nmr signals at  $\delta$ 3.68 (1H, s, C(19)-H) and 4.03 (1H, d,  $J=5.3$  Hz, C(1)- $\beta$ H) indicated the presence of C(1)-O-C(19) ether linkage in the new base<sup>4</sup>. Comparing the  $^{13}\text{C}$ -nmr data with those of napelline (5) (see table II), the appearance of a new CH signal at  $\delta$ 93.1, the disappearance of a  $\text{CH}_2$  signal at  $\delta$ 57.7, the upfield shift of C(1), the C(4) occurring downfield as the result of  $\beta$ -effect and the C(3), C(5), and C(18) occurring upfield as the result of  $\gamma$ -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<sup>7</sup> which was identical with the natural product. The reduction of this new compound with sodium borohydride produced a single product which was identical with napelline<sup>8</sup>. Therefore, the structure (1) was established.

12-Acetylucidusculine (2),  $\text{C}_{26}\text{H}_{37}\text{NO}_5$  ( $\text{M}^+$  443.2637, calc. 443.2603), mp  $132-134^\circ\text{C}$ ,  $[\alpha]_{\text{D}}^{26} -94.1^\circ$  ( $\text{C}=0.46$ ,  $\text{CHCl}_3$ ), 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  $\text{cm}^{-1}$  (exomethylene). The  $^1\text{H}$ -nmr spectrum was very similar to that of lucidusculine (6) except the appearance of an acetoxy ( $\delta$ 2.01, 3H, s) and 0.94 ppm downfield shift of C(12)- $\beta$ H. Thus, this compound was assigned to be 12-acetylucidusculine (2). Partial acetylation of lucidusculine (6) with acetic acid under the presence of trifluoroacetic acid at  $80^\circ\text{C}$  for 5 h. afforded 12-acetylucidusculine (2)<sup>3</sup> (see fig. 1). This fact confirmed the structure (2).

1-Epi-napelline (3) had mp  $87-89^\circ\text{C}$ ,  $[\alpha]_{\text{D}}^{22} -11.7^\circ$  ( $\text{C}=1.19$ ,  $\text{CH}_3\text{OH}$ ) and gave an analysis corresponding to  $\text{C}_{22}\text{H}_{33}\text{NO}_3$  ( $\text{M}^+$  359.2466, calc. 359.2460) by high resolution ms. The infrared spectrum showed absorptions at 3400 (hydroxyl), 1660 and 890  $\text{cm}^{-1}$  (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)





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  $\beta$ -orientation. Hence, this compound was deduced to be 1-epi-napelline (3). By oxidation with chromic anhydride 12-acetylucidusculine (2) yielded 1-keto-12-acetylucidusculine (17) (see fig. I) which, upon reduction with sodium borohydride, produced two products, 1-epi-12-acetylucidusculine (18) and 12-acetylucidusculine (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),  $\text{C}_{22}\text{H}_{33}\text{NO}_3$  ( $M^+$  359.2470, calc. 359.2460), mp 72-73.5°C,  $[\alpha]_D^{21}$  -40.2° (C=1.03,  $\text{CHCl}_3$ ), showed the character of napelline-type alkaloid (see table I). The double doublet of C(13)-H implied the presence of C(12)- $\alpha$ H. Irradiation at  $\delta$ 4.18 (1H, dd, J=8.8, 4.8 Hz) collapsed the signal of C(13)-H to doublet. This fact indicated that  $\delta$ 4.18 (1H, dd, J=8.8, 4.8 Hz) should be the signal of C(12)- $\alpha$ H and the C(12)- $\beta$ H must be substituted by a hydroxyl group. The mass spectrum of this compound was in consistent with that of napelline (5). Comparison of the  $^{13}\text{C}$ -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-Demethylhypoconitine (9),  $\text{C}_{32}\text{H}_{43}\text{NO}_{10}$  ( $M^+$  601.2839, calc. 601.2793), had mp 180-182°C,  $[\alpha]_D^{26}$  +19.9° (C=0.38,  $\text{CHCl}_3$ ). The infrared spectrum showed absorptions at 3500

(hydroxyl), 1725, 1290, 1150 (ester), 1605, 1589, 1490, and 713  $\text{cm}^{-1}$  (aromatic ring). The  $^1\text{H}$ -nmr spectrum in deuteriochloroform exhibited signals at  $\delta$ 1.39 (3H, s, C(8)- $\text{OCOCH}_3$ ), 2.38 (3H, s, N- $\text{CH}_3$ ), 3.15, 3.29, 3.75 (each 3H, s,  $\text{OCH}_3$ ), 7.44-8.02 (5H, m, aromatic protons), 4.87 (1H, d,  $J=4.9$  Hz, C(14)- $\beta\text{H}$ ), 3.18, 3.53 (each 1H, ABq,  $J=8.1$  Hz, C(18)-2H), 3.97 (1H, d,  $J=6.4$  Hz, C(6)- $\beta\text{H}$ ), 3.66 (1H, m, C(1)- $\beta\text{H}$ ), 3.40 (1H, d,  $J=5.2$  Hz, C(16)- $\alpha\text{H}$ ), 4.47 (1H, dd,  $J=5.4, 2.6$  Hz, C(15)- $\beta\text{H}$ ), and 4.40 (1H, d,  $J=2.6$  Hz, C(15)-OH). The  $^{13}\text{C}$ -nmr spectrum of 1-demethylhypoconitine showed twenty-eight signals for thirty-two carbon atoms in the molecule. The pattern of carbon chemical shifts was similar to that of hypoconitine (15) except ring A (see table III). The appearance of a new CH signal at  $\delta$ 71.9, disappearance of C(1) signal at  $\delta$ 85.0, 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  $\alpha$ -OH in aconitine-type alkaloids is  $\delta$ 72.0-73.0 except where a C(10)-OH or a C(2)-C(3) double bond is present<sup>10</sup>. The value for C(1) in delphinine (1-epi-neoline) with an  $\beta$ -OH is 69.0<sup>10</sup>. Since the shift for C(1) in this compound is 71.9, the C(1)-OH is assigned an  $\alpha$ -orientation. Therefore, the structure (9) was assigned to 1-demethylhypoconitine.

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