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FOUR NEW LIPO-ALKALOIDS FROM ACONITI TUBER

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Four new lipo-alkaloids, named lipoaconitine (10), lipohypaconitine (11), lipomesaconitine (12), and lipodeoxyaconitine (13), have been isolated from Aconiti Tuber ("chuanwu" imported from Si Chuan, China) and their structures elucidated on the basis of chemical and physicochemical evidence.

KEYWORDS —— Aconiti Tuber; lipo-alkaloid; lipoaconitine; lipohypaconitine; lipomesaconitine; lipodeoxyaconitine; ^{13}C NMR; ^{1}H NMR

Aconiti Tuber (Aconitum spp. tuber, Ranunculaceae) is an important ingredient of Chinese medicinal preparations which have been used as analgesic, cardiotonic, diuretic, and stimulant. Since tubers of various Aconitum spp. contain bioactive but toxic alkaloids: e.g. aconitine (1), hypaconitine (2), and mesaconitine (3), variously precessed tubers have been often used in order to avoid the hazard caused by those toxicants. In recent years, the chemical significance of those precessions has been extensively pursued, and deacetylation of the 8β-acetoxyl group with or without concomitant debenzoylation of the 14α-benzoyloxyl group of the above-mentioned alkaloids (yielding 1a, 1b, 2a, 2b, 3a, and 3b) has been generally accepted as the principal reaction(s) occurring in the precession and as responsible for the detoxication.[1)]

During the course of our studies on the chemical characterization of precession of various crude drugs, we have been working on various Aconiti Tuber (unprecessed and precessed specimens). This paper communicates the chemical characterization of the four new aconite alkaloids, lipoaconitine (10), lipohypaconitine (11), lipomesaconitine (12), and lipodeoxyaconitine (13) from Aconiti Tuber ("chuanwu" imported from Si Chuan, China).[2)] The structures of these alkaloids are noteworthy because of the possession of long-chain fatty acid residues, so we would like to give the general name "lipo-alkaloid" to these alkaloids.[3)]

Solvent fractionation followed by column chromatography (silica gel, alumina) of the methanolic extractive (prepared below 30°C) of the tuber furnished aconitine (1, 0.007% from the ext.),[4,5)] hypaconitine (2, 0.028%),[4,5)] mesaconitine (3, 0.021%),[4,5)] talatizamine (5, 0.098%),[5)] 14-acetyltalatizamine (6, 0.021%),[6)] isotalatizidine (7, 0.018%),[7)] karakoline (8, 0.019%),[7)] neoline (9, 0.016%),[7)] and four new lipo-alkaloids[3)]: lipoaconitine (10, 0.039%), lipohypaconitine (11, 0.062%), lipomesaconitine (12, 0.030%), and lipodeoxyaconitine (13, 0.050%). The identifications of known alkaloids were made by direct comparisons with authentic

samples (1, 2, 3, 9) or by comparisons of physical data with those reported for 5, 6, 7, and 8.

Lipoaconitine (10), colorless oil, $[\alpha]_D^{13}$ +6.0° ($CHCl_3$), UV λ_{max}^{EtOH} nm (ε): 227 (21000), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3495, 2922, 1721, gives three molecular ion peaks at m/z 867 (R^3 = oleoyl), 865 (R^3 = linoleoyl), 841 (R^3 = palmitoyl) in its FD-MS spectrum, while the EI-MS spectrum shows the highest ion peak at m/z 586 ($C_{32}H_{44}NO_9$,[8] $M^+-R^3O\cdot$, 6%) and the base peak at m/z 554 ($C_{31}H_{40}NO_8$, $M^+-R^3OH-CH_3O\cdot$). Treatment of 10 with 1% NaOMe-MeOH at 18°C for 6 h yielded aconine (1b), methyl benzoate, and a mixture of fatty acid methyl esters, whose composition was determined by GLC and GC-MS to be methyl linoleate (64%), methyl palmitate (20%), methyl oleate (16%), methyl stearate (<1%), and methyl linolenate (<1%).

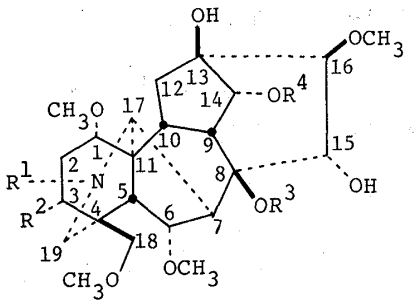
Solvolysis of 10 in dioxane-H_2O (1:1) at 120°C for 1 h[9] furnished benzoylaconine (1a) in 81% yield. Thus, the 14α-benzoyloxyl group in 10 has been proved and the fatty acid residues have been presumed to attach to 8β-OH. The presumption has been supported by the MS fragmentation pattern[7] and ^1H and ^{13}C NMR analyses[10,11] (Table I).

Furthermore, treatment of aconitine (1) with an equimolar mixture of pyridine and linoleic acid at 70°C for 4 h furnished in 84% yield 8-O-linoleoyl-benzoylaconine (10a), $C_{50}H_{75}NO_{11}$,[12] colorless oil, m/z 865 (M^+, FD-MS), $[\alpha]_D^{19}$ +20.4°($CHCl_3$), UV (EtOH): 228 (21000), IR ($CHCl_3$): 3505, 2910, 1723. 10a was obtained in the same yield by heating 1 either in an equimolar mixture of collidine and linoleic acid or with sodium linoleate in tetrahydrofuran. However, similar treatment of 1a did not afford 10a. The linoleate (10a) synthesized here is equivalent to the major ingredient of 10 and shows identical behavior with 10 on TLC and HPLC.

Based on the above evidence together with the examination of the ^{13}C NMR data for 1, 10, and 10a (Table I), the structure of 10 including the location and the composition of the fatty acid residues has been established. The above conversion from 1 to 10a has shown that the 8β-OAc group is readily replaced by 8β-OR (R= a fatty acid residue) presumably *via* trans-esterification.

The structures of other lipo-alkaloids have been clarified on the same basis. On 1% NaOMe-MeOH treatment, lipohypaconitine (11), colorless oil, $[\alpha]_D^{22}$ +13.5° ($CHCl_3$), UV (EtOH): 227 (17000), IR ($CHCl_3$): 3511, 2929, 1718, EI-MS (m/z, %): 556 ($C_{31}H_{42}NO_8$, $M^+-R^3O\cdot$, 1), 524 ($C_{30}H_{38}NO_7$, $M^+-R^3OH-CH_3O\cdot$, 100), FD-MS (M^+, m/z): 837 (R^3 = oleoyl), 835 (R^3 = linoleoyl), 811 (R^3 = palmitoyl), liberated hypaconine (2b), methyl benzoate, and fatty acid methyl esters [GC-MS: methyl linoleate (58%), methyl palmitate (19%), methyl oleate (23%), methyl stearate (<1%), methyl linolenate (<1%)]. Solvolysis[9] of 11 yielded benzoylhypaconine (2a), while heating 2 with pyridine-linoleic acid gave 11a, $C_{49}H_{73}NO_{10}$, colorless oil, $[\alpha]_D^{19}$ +13.0° ($CHCl_3$), UV (EtOH): 228 (17000), IR ($CHCl_3$): 3511, 2929, 1718, FD-MS: 835 (M^+).

On 1% NaOMe-MeOH treatment, lipomesaconitine (12), colorless oil, $[\alpha]_D^{13}$ +13.8° ($CHCl_3$), UV (EtOH): 227 (18700), IR ($CHCl_3$): 3495, 2924, 1714, EI-MS: 572 ($C_{31}H_{42}NO_9$, $M^+-R^3O\cdot$, 4), 540 ($C_{30}H_{38}NO_8$, $M^+-R^3OH-CH_3O\cdot$, 100), FD-MS (M^+): 853 (R^3 = oleoyl), 851 (R^3 = linoleoyl), 827 (R^3 = palmitoyl), liberated mesaconine (3b), methyl benzoate, and fatty acid methyl esters [GC-MS: methyl linoleate (57%), methyl palmitate (32%), methyl oleate (11%), methyl stearate (<1%), methyl linolenate (<1%)]. Solvolysis[9] of 12 gave benzoylmesaconine (3a), while pyridine-linoleic acid treatment of 3 yielded 12a, $C_{49}H_{73}NO_{11}$, colorless oil, $[\alpha]_D^{19}$ +15.0°



1 : $R^1=C_2H_5$, R^2=OH, R^3=Ac, R^4=Bz
1a : $R^1=C_2H_5$, R^2=OH, R^3=H, R^4=Bz
1b : $R^1=C_2H_5$, R^2=OH, $R^3=R^4$=H

2 : $R^1=CH_3$, R^2=H, R^3=Ac, R^4=Bz
2a : $R^1=CH_3$, $R^2=R^3$=H, R^4=Bz
2b : $R^1=CH_3$, $R^2=R^3=R^4$=H

3 : $R^1=CH_3$, R^2=OH, R^3=Ac, R^4=Bz
3a : $R^1=CH_3$, R^2=OH, R^3=H, R^4=Bz
3b : $R^1=CH_3$, R^2=OH, $R^3=R^4$=H

4 : $R^1=C_2H_5$, R^2=H, R^3=Ac, R^4=Bz
4a : $R^1=C_2H_5$, $R^2=R^3$=H, R^4=Bz
4b : $R^1=C_2H_5$, $R^2=R^3=R^4$=H

10 : $R^1=C_2H_5$, R^2=OH, R^3=linoleoyl, palmitoyl, oleoyl, stearoyl, linolenoyl, R^4=Bz
10a : $R^1=C_2H_5$, R^2=OH, R^3=linoleoyl, R^4=Bz

11 : $R^1=CH_3$, R^2=H, R^3=linoleoyl, palmitoyl, oleoyl, stearoyl, linolenoyl, R^4=Bz
11a : $R^1=CH_3$, R^2=H, R^3=linoleoyl, R^4=Bz

12 : $R^1=CH_3$, R^2=OH, R^3=linoleoyl, palmitoyl, oleoyl, stearoyl, linolenoyl, R^4=Bz
12a : $R^1=CH_3$, R^2=OH, R^3=linoleoyl, R^4=Bz

13 : $R^1=C_2H_5$, R^2=H, R^3=linoleoyl, palmitoyl, oleoyl, stearoyl, linolenoyl, R^4=Bz

Table I. ^{13}C NMR Data (in $CDCl_3$)[a)]

Carbon	1[11)]	10	10a	11	11a	12	12a	13
1	83.4	83.2(d)	83.5(d)	84.8(d)	85.0(d)	83.1(d)	83.3(d)	85.0(d)
2	36.0	35.6(t)	35.8(t)	26.1(t)	26.3(t)	35.6(t)	35.8(t)	26.2(t)
3	70.4	70.4(d)	70.4(d)	34.7(t)	34.8(t)	70.5(d)	71.0(d)	35.1(t)
4	43.2	42.9(s)	43.2(s)	39.0(s)	39.3(s)	43.3(s)	43.5(s)	39.0(s)
5	46.6	46.0(d)	46.4(d)	49.8(d)	50.1(d)	46.0(d)	46.1(d)	49.0(d)
6	82.3	82.1(d)	82.4(d)	83.0(d)	83.3(d)	82.3(d)	82.3(d)	83.2(d)
7	44.8	44.5(d)	44.8(d)	44.4(d)	44.7(d)	44.2(d)	44.4(d)	45.0(d)
8	92.0	91.4(s)	91.7(s)	91.3(s)	91.6(s)	91.3(s)	91.5(s)	91.6(s)
9	44.2	44.0(d)	44.4(d)	43.7(d)	44.1(d)	43.6(d)	43.9(d)	44.5(d)
10	40.8	40.6(d)	41.0(d)	40.9(d)	41.2(d)	40.7(d)	40.9(d)	40.9(d)
11	49.8	49.7(s)	50.1(s)	49.8(s)	50.1(s)	49.9(s)	50.2(s)	49.9(s)
12	34.0	34.4(t)	34.8(t)	34.5(t)	34.8(t)	34.0(t)	33.8(t)	36.5(t)
13	74.0	73.8(s)	74.1(s)	73.9(s)	74.1(s)	73.9(s)	74.1(s)	74.0(s)
14	78.9	78.7(d)	79.0(d)	78.8(d)	79.0(d)	78.8(d)	79.0(d)	78.8(d)
15	78.9	78.7(d)	79.0(d)	78.8(d)	79.0(d)	78.8(d)	79.0(d)	78.9(d)
16	90.1	89.8(d)	90.1(d)	90.0(d)	90.2(d)	90.0(d)	90.1(d)	90.1(d)
17	61.0	60.9(d)	61.2(d)	61.9(d)	62.3(d)	62.0(d)	62.4(d)	61.0(d)
18	75.6	75.7(t)	75.6(t)	79.8(t)	80.1(t)	75.5(t)	76.1(t)	80.0(t)
19	48.8	48.6(t)	48.9(t)	49.8(t)	50.1(t)	49.2(t)	49.6(t)	53.1(t)
N-$CH_{2(3)}$	46.9	46.7(t)	47.1(t)	42.3(q)	42.6(q)	42.2(q)	42.4(q)	48.6(t)
CH_3	13.3	13.0(q)	13.3(q)					13.2(q)
1-OCH_3	55.7	55.5(q)	55.8(q)	56.2(q)	56.1(q)	56.1(q)	56.5(q)	56.0(q)
6-OCH_3	57.9	57.8(q)	58.2(q)	57.8(q)	58.1(q)	58.0(q)	58.1(q)	58.0(q)
16-OCH_3	60.7	60.5(q)	60.9(q)	60.8(q)	61.1(q)	60.9(q)	61.2(q)	60.9(q)
18-OCH_3	58.9	58.7(q)	59.0(q)	58.7(q)	59.0(q)	58.8(q)	59.1(q)	58.8(q)
8-O·COR	172.2	174.7(s)	175.1(s)	174.7(s)	175.1(s)	174.9(s)	175.1(s)	174.8(s)
14-O·COPh	165.9	165.6(s)	166.0(s)	165.6(s)	166.0(s)	165.8(s)	166.0(s)	165.8(s)

a) Assignments for C-7 and C-9 may be interchangeable in the same vertical column.

($CHCl_3$), UV (EtOH): 228 (18000), IR ($CHCl_3$): 3500, 2931, 1719, FD-MS: 851 (M^+).

On 1% NaOMe-MeOH treatment, lipodeoxyaconitine (13), colorless oil, $[\alpha]_D^{13}$ +12.4° ($CHCl_3$), UV (EtOH): 227 (16800), IR ($CHCl_3$): 3503, 2925, 1717, EI-MS: 570 ($C_{32}H_{44}NO_8$, $M^+-R^3O\cdot$, 2), 538 ($C_{31}H_{40}NO_7$, $M^+-R^3OH-CH_3O\cdot$, 100), FD-MS (M^+): 851 (R^3 = oleoyl), 849 (R^3 = linoleoyl), 825 (R^3 = palmitoyl), liberated deoxyaconine (4b), methyl benzoate, and fatty acid methyl esters [GC-MS: methyl linoleate (61%), methyl palmitate (19.5%), methyl oleate (19.5%), methyl stearate (<1%), methyl linolenate (<1%)]. Solvolysis[9] of 13 gave benzoyl-deoxyaconine (4a). Together with these findings, comparative examination of the physicochemical properties (including the ^{13}C NMR data) of deoxyaconitine (4) and 13 has led to the formulation of lipodeoxyaconitine as 13.

After chemical characterization of the above-described four lipo-alkaloids, we have examined the alkaloidal constituents of several other specimens of Aconiti Tuber (precessed) which are available in the Osaka market and we have also examined some biological activities of these lipo-alkaloids.[14] The results will be published in our forthcoming paper.

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2) Aconiti Tuber analyzed in this paper was purchased from Mikuni Co. Osaka. The botanical origin was said to be the unprecessed tuber of Aconitum carmichaeli Debx. However, as is apparent from the present study, the morphological inspection of the tuber seems to be necessary.
3) I. Kitagawa, Z. L. Chen, and M. Yoshikawa, presented at the Annual Meeting of the Japanese Society of Pharmacognosy, held in Tokyo, Oct. 2-3, 1981. Abstract Papers, p. 51. The four lipo-alkaloids described here are respectively mixtures of alkaloids having different types of fatty acid residues, although each lipo-alkaloid behaved as a single compound on TLC, GLC, and HPLC[4,6,13] and the ^{13}C and ^1H NMR spectra of each lipo-alkaloid show readily assignable signals due to the alkaloidal moiety. Although separation of the respective ingredients was not yet successful, we designate them for convenience as described in this paper.
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