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AMBIGUINE AND DIHYDROAJACONINE: TWO NEW DITERPENOID ALKALOIDS FROM CONSOLIDA AMBIGUA[1]

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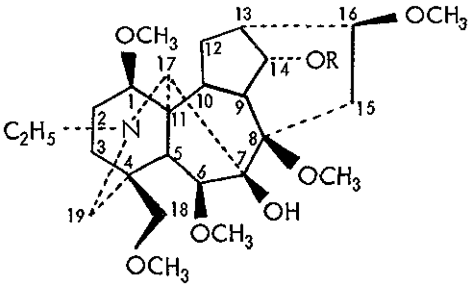
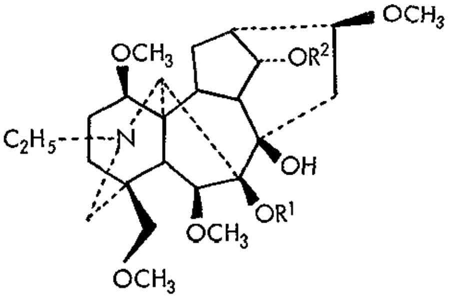
Chemical investigation of the minor alkaloids of Consolida ambigua has led to the isolation and characterization of two new diterpenoid alkaloids, ambiguine (1) and dihydroajaconine (7). Ambiguine is the first lycoctonine-type alkaloid in which a methoxy group is present at the C(8)-position, and dihydroajaconine is a reduced form of the known alkaloid, ajaconine.

Consolida ambigua[1] is an ornamental plant which is commonly known as garden larkspur. Some of the Consolida (earlier reported as Delphinium) plants are known to possess insecticidal and growth inhibiting activities.[2] Recently, we have reported[3] the structures of two new alkaloids, ajacusine and ajadine, isolated from the seeds of garden larkspur.[1] Further work on this plant has resulted into the isolation of two new minor alkaloids, ambiguine and dihydroajaconine. In this communication, we report the structures of ambiguine (1) and dihydroajaconine (7) by the aid of ^{13}C nmr spectroscopy.

Ambiguine, $C_{28}H_{45}NO_8$ (element analysis and MS), mp 106-108° (corrected), $[\alpha]_D^{25}$ + 38° (c 1.0, $CHCl_3$) was isolated from the 85% ethanol extract of the seeds by a combination of gradient pH separation, thick layer, and column chromatographic techniques. Infrared absorption at 3560, 1735 and 1090 cm^{-1} indicated the presence of hydroxyl, acetate, and ether groups, respectively. The ^1H nmr spectrum of ambiguine in $CDCl_3$ exhibited the presence of an $N\text{-}CH_2\text{-}C\underline{H}_3$ (3H, t, J 7.5 Hz) centered at δ 1.03, $-OCOC\underline{H}_3$ group (3H, s) at δ 2.05 and 5 aliphatic methoxy groups (each 3H, s) at δ3.28, 3.35, 3.38, 3.48 and 3.55. A doublet of doublets observed at δ 4.72 is typical of a C(14) β-proton when an α-hydroxy group at C(14) is esterified. On the basis of these data and the nature of other C_{19}-diterpenoid alkaloids isolated from C. ambigua, we conclude that ambiguine has a lycoctonine-type skeleton. Alkaline hydrolysis of ambiguine (5% KOH in methanol) gave the aminoalcohol (2) which on acetylation (Ac_2O/ pyridine) regenerated ambiguine. Treatment of ambiguine with Ac_2O/BF_3 or Ac_2O/ p-toluenesulfonic acid did not acetylate the tertiary C(7)-hydroxy group, probably because of steric factors. The fragmentation pattern of ambiguine in the mass spectrum also indicated the presence of a lycoctonine-type skeleton. The base peak at $M^+ -31$ in the mass spectrum revealed the presence of a methoxy group at C(1).[4]





1 R = Ac Ambiguine
2 R = H

3 $R^1 = R^2 = CH_3$
4 $R^1 = H$; $R^2 = CH_3$ Delphatine
5 $R^1 = R^2 = H$ Browniine
6 $R^1 = H$; $R^2 = Ac$

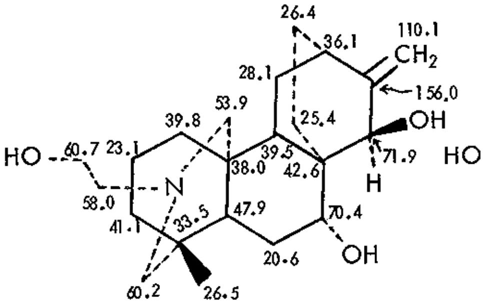
The ^{13}C nmr spectrum of ambiguine is consistent with structure 1. The chemical shifts of the 28-carbons in the molecule have been assigned (Table 1) on the basis of noise decoupled spectra, direct analysis of non-protonated carbons, single-frequency off-resonance decoupling (SFORD) techniques and by comparison with the ^{13}C nmr spectra of closely related alkaloids.[5] The signal at 75.0 ppm supports the presence of a C(14)-

acetyl group. The chemical shift does not change (74.9 ppm) when the alkaloid is hydrolyzed to the aminoalcohol (2). We have observed[5] the same behavior with several known C_{19}-diterpenoid alkaloids having an α - C(14)-acetate group. Comparison of the ^{13}C nmr spectra of ambiguine with that of its hydrolyzed product (2) demonstrated the shielding (1.6 ppm) effect of the C(14)-acetate on the chemical shift of C(17) (Table 1). A similar effect has been observed in case of browniine (5) and its 14-acetate (6).

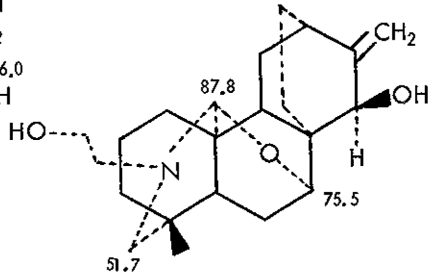
In order to exclude the possibility of a methoxy group at C(7) in ambiguine, the ^{13}C nmr spectra of some model compounds were required. For this purpose, we have prepared the known compounds O,O dimethyllycoctonine (3) and delphatine (4) from lycoctonine by methylation with CH_3I and NaH at 110° for 26 hr. Correlation of compounds 3 through 6 with ambiguine and its hydrolyzed derivative was made through a study of their carbon-13 nmr spectra (Table 1). This comparison afforded evidence for the presence of a methoxyl group at C(8) as well as the basic lycoctonine-type skeleton in ambiguine. These ^{13}C nmr studies indicate that ambiguine is similar to 14-acetylbrowniine except for the presence of a methoxyl group at C(8). The carbon-13 chemical shifts of ambiguine are in agreement with its structure (1). Although over twenty-five lycoctonine-type alkaloids are well known in nature, ambiguine is the first to bear a methoxyl group at C(8).

Dihydroajaconine, $C_{22}H_{35}NO_3$, mp 99-100° C, $[\alpha]_D^{24}$ - 35° (c 1.0 Abs. EtOH), was isolated from the mother liquors accumulated during the isolation of ajaconine from garden larkspur. The ir spectrum in KBr exhibited peaks at 3350 cm^{-1} (OH), 1660 and 920 ($>C=CH_2$). The ^1H nmr spectrum of dihydroajaconine in $CDCl_3$ revealed the presence of a singlet at δ 0.80 for the C(4)-methyl group and a multiplet centered at δ 5.12 for the exocyclic methylene group.

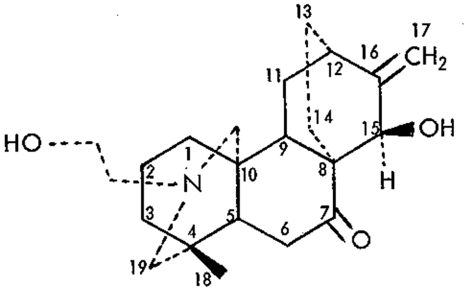
The ^{13}C nmr spectrum of dihydroajaconine (7) showed the presence of one methyl group, eleven methylene carbons, five methine carbons, four quarternary carbon atoms, and two olefinic carbons. Comparison of the ^{13}C nmr spectrum of dihydroajaconine with that of ajaconine (8)[6] indicated that the only difference between these two alkaloids was the presence of a C(7)-hydroxyl group in the α-configuration in 7 instead of a C(7)-C(20) carbinolamine ether linkage. Unambiguous carbon signal assignment in dihydroajaconine (7) was achieved by using conventional techniques, additivity relationships, a direct analysis of non-protonated carbon centers and by comparison with the ^{13}C nmr spectra of closely related alkaloids[7], e.g. atidine, ajaconine.



7 Dihydroajaconine



8 Ajaconine



9 Atidine

Reduction of ajaconine with sodium borohydride in aq. methanol afforded, in a quantitative yield, a product which was identical with natural dihydroajaconine. This correlation established the complete structure of the new alkaloid as 7. To our knowledge, dihydroajaconine has not previously been isolated as a natural product.[8]

Biogenetically, dihydroajaconine may be an important precursor between ajaconine (8) and atidine (9)[9].

Table 1. Carbon-13 Chemical Shifts and Assignments for Ambiguine and Related Alkaloids

Carbon	**1**	**2**	**3**	**4**	**5**	**6**
1	83.4	83.9	84.3[c]	83.9[c]	85.2	84.2
2	25.3	24.9	26.2	26.2	25.5	26.2
3	31.5	31.7	32.5	32.4	32.5	32.4
4	38.3	38.6	38.1	38.1	38.4	38.1
5	40.9[e]	42.7[e]	44.6[e]	43.3[e]	45.1[e]	42.6[e]
6	91.1	91.0	85.1	90.6	90.1	90.3
7	90.0	89.9	92.8	88.4	89.1	88.3
8	80.4	79.6	80.0	77.5	76.3	77.1
9	52.0	52.6	50.5	49.8	49.6	51.2
10	35.9	36.6	39.0	38.1	36.4	38.1
11	47.2	46.4	49.0	48.9	48.2	49.5
12	27.4	27.6	29.0	28.7	27.5	28.2
13	46.1[e]	46.7[e]	45.7[e]	46.1[e]	46.1[e]	45.7[e]
14	75.0	74.9	84.7[c]	84.3[c]	75.3	76.0
15	28.5	26.8	33.5	33.5	33.1	33.7
16	81.9	82.3	82.9	82.6	81.7	82.4
17	66.7	68.3	66.5	64.8	65.4	64.8
18	79.3	79.1	78.2	78.1	78.0	78.0
19	53.6	53.9	52.3	52.8	52.7	52.7
N-CH_2	52.6	52.9	51.5	51.1	51.3	48.8
CH_3	14.9	15.3	14.0	14.2	14.3	14.2
1'	55.6	55.7	55.8[d]	55.7	56.0	55.8
6'	59.4	59.2	56.0[d]	57.3	57.5	57.3
7'	-	-	55.5	-	-	-
8'	53.7	54.0	-	-	-	-
14'	-	-	57.8	57.8	-	-
16'	56.3	56.5	56.3	56.3	56.5	56.2
18'	59.4	59.5	59.1	59.0	59.1	59.0

Carbon	**1**	**2**	**3**	**4**	**5**	**6**
$-C=O$	171.3	-	-	-	-	171.9
CH_3	21.4	-	-	-	-	21.5

[a] Chemical shifts in ppm downfield from TMS. Solvent deuterochloroform.

[b] Spectra were taken at 15.03 MHz in the Fourier mode using a FX-60 spectrometer in conjunction with a JEC-980 computer.

[c,d] These assignments may be interchanged in any vertical column.

[e] These assignments may be interchanged in any vertical column. At the present we do not have model compounds with a C(13)-hydroxyl or methoxyl group to confirm the assignments.

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8 Sastry and Waller (Chem. and Ind., 1972, 381) reported the presence of dihydroajaconine in GC-MS studies of ajaconine isolated from Delphinium ajacis. A sample of what had been earlier identified as pure ajaconine furnished a mixture of five components when the deuterated trimethylsilyl derivative was analysed on the GC-MS. The temperature on the GC column (215°C) and the time required for elution (12.7-27.8 min.) may have been responsible for the rearrangement products. However, the authors believed their evidence demonstrated that the ajaconine sample was a mixture of five components.

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