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# THE STRUCTURE OF THE ACONITE ALKALOID PANICULATINE [1]

A. Katz* and E. Staehelin

Laboratory of the Holbein-Apotheke
Oberwilerstr. 7, CH-4054 Basel, Switzerland

**Abstract:** Based on the analysis of UV-, M- and NMR-spectra we established structure (1) for paniculatine, showing an atisine type skeleton, which is 1β, 2β-diacetoxy, 11α-hydroxy, 13β-benzoyloxy substituted and which, like hetisine, has C(20)-C(14) and N-C(6) bonds.

In a previous paper[2] we reported the isolation of the alkaloid paniculatine (1) from the tuber of a chemovariety of Aconitum paniculatum Lam., which we found to grow in a defined part of the western Alps. It was isolated from this species as early as 1921 by G. E. Brunner[3], but has not been much considered since. It's existence has even been doubted[4]. On the bases of IR-, $^{13}$C-, $^{1}$HNMR- and M-spectra we put forth the following findings: Molecular weight 533, mol. formula $C_{31}H_{35}NO_7$, 7 rings; 1 hydroxy, 1 benzoyloxy, 2 acetoxy groups (all four on methine carbon); 1 C-$CH_3$, 1 double bond (exocyclic $CH_2$) and 1 tertiary N. We tentatively posited that paniculatine is a diterpenoid alkaloid of the hetisine[5,6] type, a subtype of the atisine type[7]. In the present paper we wish to report the basic facts, which led us to the formulation (1) for paniculatine.

Acetoxy groups:

$^{13}$CNMR: δ 21,3 and 21,8 ppm ($OCO\underline{C}H_3$)
δ 170,1 and 171,4 ppm ($O\underline{C}OCH_3$)

$^{1}$HNMR: δ 5,84 ppm, d, J = 3 Hz (H-C(1))
δ 5,55 ppm, m, $w\frac{1}{2}$ = 9 Hz (H-C(2))
δ 1,8 - 1,95 ppm, m ($H_2$-C(3))

MS: $M^+$ 533, m/e 474 (m* 422), m/e 414 (m* 362)

In the double resonance spectrum the signal δ 5,84 ppm appears as a singlet when δ 5,55 ppm is irradiated. Simultaneously the multiplet δ 1,8 - 1,95 ppm is affected. Irradiation at δ 1,9 ppm affects the signal δ 5,55 ppm. Hence we can formulate the sequence -C-$HCOCOCH_3$-$HCOCOCH_3$-$CH_2$-C- which, in the proposed ring system, only fits ring A. In the Nuclear Overhauser Effect experiment the signals of H-C(20) (δ4,30 ppm; cf. N-substituted groups) and of HO-C(11) (δ1,63 ppm; cf. Hydroxy group) appear when the proton with the signal δ 5,84 ppm is saturated. This shows it's position at C(1), as well as it's α-configuration. Concomitantly the signal of the adjacent $\underline{H}$-$COCOCH_3$ appears, to which therefore α-position may be assigned too. Consequently ring A is 1β, 2β-diacetoxy substituted. The small coupling constants prove it to have boat conformation. The mass spectrum confirms the 1,2-position of the acetoxy groups: Yunusov et al.[8] found that in the mass spectrum of aconitine type alkaloids, which have a 1-acetoxy group, the 100%-peak is formed by ejection of $C_2H_3O_2^+$. In accordance with this the 100%-peak in paniculatine is m/e 474, which originates from the molecular ion by loss of $C_2H_3O_2^+$ (m* calc. 421,5 for $M^+$-59). In a second step ejection of $CH_3COOH$ from C(2) causes an intensive peak m/e 414 (m* calc. 361,6 for m/e 474 - 60).

**Hydroxygroup:**

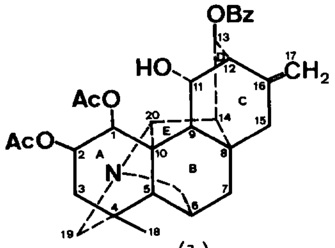
| | Paniculatine | Dehydropaniculatine | Assignment |
|---|---|---|---|
| $^{13}$CNMR: | $\delta$ 75,9 ppm, d | ------ | H-$\underline{C}$(11)-OH |
| | ------ | $\delta$ 206,7 ppm, s | O=$\underline{C}$(11) |
| $^{1}$HNMR: | $\delta$ 4,19 ppm, m<br>$w_{\frac{1}{2}}$ = 16 Hz | ------ | $\underline{H}$-C(11)-OH |
| | $\delta$ 1,63 ppm | ------ | H-C(11)-O$\underline{H}$ |
| MS: | $M^+$ 533 | $M^+$ 531 | |
| UV: | ------ | 300 Å (log $\epsilon$ = 2,5) | $H_2C(17){=}C(16){-}HC(12){-}\overset{O}{\overset{\Vert}{C}}(11){-}$ |

Dehydration of paniculatine with $CrO_3$ yields a monoketone which shows in the UV an absorption-maximum at 3000Å (log $\epsilon$ = 2,5) which indicates a $\beta,\gamma$-unsaturated ketone. As the only double bond in the polycyclic system is that of the exocyclic methylene at C(16), the possible positions of the ketogroup are C(11) or C(13). In paniculatine itself we expect therefore $\underline{H}$-COH to couple with H-C(12) and with H-C(9) or H-C(14). As the signals of these three protons are crowded and overlapping, and as furthermore the structure of the rings C, D and E is almost symmetrical (cf. ($\underline{2}$)), the assignments are difficult to be made even when applying double resonance technique.

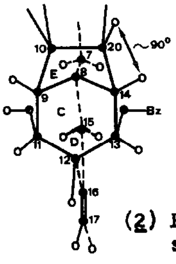
**Benzoyloxygroup:**

$^{13}$CNMR: $\delta$ 128,6, 129,9, 130,3, 133,0 ppm ($\underline{C}_6H_5COO-$); 165,5 ppm ($C_6H_5$-$\underline{C}$OO-)
$^{1}$HNMR: $\delta$ 7,46 ppm, t; 7,58 ppm, t; 8,13 ppm, d ($C_6H_5$-COO-)
$\delta$ 5,37 ppm, m, 1 H (H-C(13))

The signal $\delta$ 5,37 ppm necessarily is caused by the proton of the fourth oxygen substituted carbon, i. e. by $\underline{H}$-COO-$C_6H_5$. Like $\underline{H}$-C(OH) it couples with the complex agglomeration of the signals of H-C(12), H-C(9) and H-C(14). Again we can not make a decision on the basis of the spectral data given above. The answer came from the Nuclear Overhauser Effect experiment: When H-C(20) ($\delta$4,30 ppm) is saturated, the signal of the ortho-proton of the benzoyloxygroup appears ($\delta$8,13 ppm). From the Dreiding model it can be deduced that this again includes both possibilities, C(11) and C(13). In the first case however, and only in the first case, the signal of the ortho-proton also should show up, when H-C(9) or H-C(13) is saturated. This is not the case. In the second case the benzoyloxygroup can take a position which brings the o-proton close only to



($\underline{1}$)



($\underline{2}$) Rings C, D, E seen from "above", slightly inclined.

the C(20)-proton (2,3 Å)[*]. Hence the benzoyloxy group has to be placed at C(13) and consequently the hydroxyl at C(11).

The configuration of the hydroxy- and of the benzoyloxy groups could be determined through the NOE-experiment. When H-C(1) is saturated, the hydroxyl proton appears. This proves the C(11)-hydroxy group to be $\alpha$-orientated (minimal distance $1\alpha$-H/$11\alpha$-O$\underline{H}$ = 1,3 Å; $1\alpha$-H/$11\beta$-H = 4,0 Å). On the other hand the hydroxyl proton does not show up when H-C(13) is saturated, which enables us to deduce the $\beta$-position of the benzoyloxy group at C(13) (distance $13\alpha$-H/$11\alpha$-O$\underline{H}$ = 3,7 Å; $13\beta$-H/$11\alpha$-O$\underline{H}$ = 2,3 Å).

<u>N-substituted groups:</u>

$^1$HNMR: $\delta$ 4,30 ppm, s, 1 H (H-C(20))
$\delta$ 3,29 ppm, m, 1 H, $w\frac{1}{2}$ = 7 Hz (H-C(6))
$\delta$ 2,51 and 2,88 ppm, AB-system, $J_{AB}$= 15 Hz, $\Delta\delta_{AB}$= 145 Hz ($H_2$C(19))

H-C(20) appears as a singlet, the dihedral angle with the only adjacent proton (H-C(14)) being almost 90°. In the double resonance spectrum no significant coupling can be observed. The multiplet of H-C(6) is a broad single peak which couples with the multiplet of C(7). Since $H_2$-C(19) has no neighbouring protons and does not show long range coupling, it appears as a pure AB-system.

<u>Exocyclic methylene group:</u>

$^{13}$CNMR: $\delta$ 144,3 ppm, s (-$\underline{C}$(16)=C(17)$H_2$)
$\delta$ 109,0 ppm, t (-C(16)=$\underline{C}$(17)$H_2$)
$^1$HNMR: $\delta$ 4,90 and 4,77 ppm ($H_2$-C(17)=)
UV: cf. keto group in dehydropaniculatine

NMR-signals indicate the presence of an exocyclic methylene group on a ring carbon. On the basis of literature data[5,9,1] we may assign it to C(16).

<u>High field $^1$HNMR-signals (<2,5 ppm):</u>

$^1$HNMR: $\delta$ 2,35 and 2,08 ppm, AB-system, $J_{AB}$= 20 Hz, $\Delta\delta_{AB}$= 100 Hz ($H_2$-C(15))
$\delta$ 2,32, 2,29, 2,27, 2,26 ppm (H-C(9), H-C(12), H-C(14))
$\delta$ 2,07 ppm, s (H-C(5))
$\delta$ 2,03 ppm, s (2 x -OCOC$H_3$)

The signals of $H_2$-C(15) do not represent a pure AB-system as they show long range coupling with $H_2$-C(17). They are strongly superimposed by the signals of H-C(9), H-C(12) and H-C(14) as well as with those of H-C(5) and of the acetoxygroups. The signals of H-C(9), H-C(12) and H-C(14) occur in a complex agglomeration in which we were unable to make exact assignments. In the double resonance spectrum, however, coupling with H-C(11) and H-C(13) could be recognized. H-C(5) appears as a singlet because its dihedral angle with H-C(6) is 90°.

$^1$HNMR: $\delta$ 1,97 - 1,78 ppm, m ($H_2$-C(3))
$\delta$ 1,83 - 1,63 ppm, m ($H_2$-C(7))

$H_2$-C(3) and $H_2$-C(7), both form complex multiplets which, moreover, are overlapping. The first

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[*] This means that the benzoyloxy group has a fixed position.

mentioned couples with H-C(2), the second one with H-C(6).

$^1$HNMR: $\delta$ 1,63 ppm, broad s (HO-C(11))

This signal is lacking in the deuterated spectrum, as well as in dehydropaniculatine. It therefore is to be attributed to the hydroxyl proton at C(11).

All the given data are compatible with the proposed formula, whereas, when other structures are considered, results will be self contradictory. We therefore assign structure (1) to paniculatine. It shows a hetisine ring system which is 1$\beta$, 2$\beta$-diacetoxy, 11$\alpha$-hydroxy, 13$\beta$-benzoyloxy substituted. Ring A is boat shaped. A threefold esterified $C_{20}$-diterpenoid alkaloid is unusual. In 1971 Tel'nov et al.[10] isolated from the russian Aconitum anthoroideum an alkaloid which they called anthoroidine. A similar melting point, the same molecular formula and the presence of one C-methyl, one benzoyloxy and two acetoxy groups, as well as of one double bond suggest possible identity with paniculatine. We kept Brunner's name paniculatine which dates from 1921[3].

## Experimental Part:

Dehydropaniculatine was prepared by $CrO_3$ oxydation of paniculatine in conc. acetic acid (2 h, room temp.). Crude oxydation product purified by TLC. Yield 50 %. Amorphous. $R_F$ = 0,39 ($R_F$ of paniculatine = 0,45) (Plate: HPTLC Merck silicagel 60 $F_{254}$, Linear Developing Chamber Camag, 10 min. of saturation, front: 7 cm, solvent: Cyclohexane-chloroform-diethylamine 5:4:1).

$^{13}$CNMR-spectra: 90,5 Hz; 30 mg subs./500 $\mu$l $CDCl_3$; ref. TMS.

$^1$HNMR-spectra: 360 Hz; 5 mg subs./400 $\mu$l $CDCl_3$; ref. TMS.

UV-spectrum: Solvent: Ethanol.

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