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12-EPINAPELLINE AND ITS N-OXIDE FROM *Aconitum baicalense*

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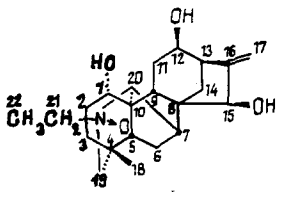
12-Epinapelline and its N-oxide have been isoated from the epigeal part of Aconitum baicalense *Turcz. ex Rapaics (A. Czekanovskyi Steinb.). The structures of these compounds have been determined with the aid of two-dimensional ^1H and ^{13}C NMR spectroscopy, and IR and mass spectrometry.*

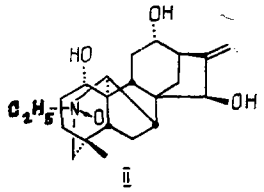
Diterpene alkaloids belonging to the C_{19} (mesaconitine, hypaconitine) and C_{20} (napelline, songorine) types have previously been isolated from *Aconitum baicalense* Turcz. ex Rapaics (A. Czekanovskyi Steinb.) [1]. Investigating the minor terpene bases of the plant, we have chromatographed on alumina the chloroform-soluble fraction of the total alkaloids and have isolated a base (I).

The molecular mass of the alkaloid was determined as 375 a.m.u., and its elementary composition as $C_{22}H_{33}O_4N$. Its IR spectrum showed an absorption band at 905-920 cm^{-1}, which gave grounds for assuming the presence of a N-oxide functional group. It followed from a consideration of ^{13}C and ^1H NMR spectra of the alkaloid that its molecule also included three oxygen-containing functions, an exomethylene double bond, a N-ethyl group and a C-methyl group, the latter being attached to a tertiary carbon atom. On the basis of these facts we assumed that the compound had the carbon skeleton of napelline. A more detailed study of the NMR spectra at a working frequency of 500 MHz using double-resonance procedures (COSY, HETCOR) permitted us to put forward for the alkaloid the structure of 12-epinapelline N-oxide (I).

The spectral characteristics of base (I) were close to those of flavamine (II) [2]. The main differences were observed for the C-12, C-13, C-16, and C-17 atoms (Table 2). From this it was possible to conclude that the alkaloids under consideration differed by the stereochemictry at the C-12 atom. In actual fact, in the PMR spectrum the H-13 signal at 2.80 ppm consisted of a doublet of doublets with $J_{12,\ 13}$ = 8.7 Hz, $J_{13,\ 14a}$ = 4.1 Hz and $J_{13,\ 14e}$ = 0 Hz. The dihedral angles corresponding to these SSCCs presuppose the axial orientation of the hydroxy group at C-12, as can be seen from formula (IV). In flavamine, the H-13 signal has the form of a doublet with a SSCC of 3.9 Hz [2]. The spin-spin interactions of the H-12 proton in alkaloid (I) also correspond to the stereochemistry (IV).



I



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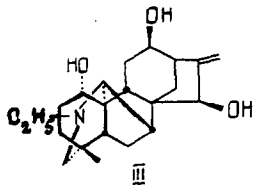
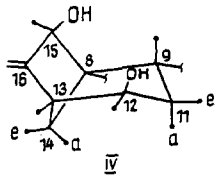


III



IV

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TABLE 1. Parameters of the PMR Spectra of Alkaloids (I) and (III)

H-atom	Chemical shift (δ, ppm); multiplicity, SSCC (Hz)	
	I*	III**
1	3.86; t; 7.1	3.90; dd; 8.2; 6.1
2e	2.45; m	1.99; m
2a	1.95; m	1.81; m
3e	1.95; m	1.61; m
3a	1.30; m	1.35; m
5	1.50; br.d; ~7.9	1.35; m
6e	2.71; dd; 7.9; 14.0	2.31; dd; 13.4; 7.6
6a	1.30; m	1.35; m
7	2.02; d; 5.3	2.04; d; 5.3
9	2.08; dd; 6.5; ~13.0	1.81; dd; 6.5; ~13.0
11e	2.25; ddd; 15.0; 12.9; 6.0	2.11; m
11a	1.70; dd; 15.0; 6.5	1.61; m
12	4.18; dd; 8.7; ~6.0	4.14; dd; ~9.0; 4.7
13	2.80; dd; 8.7; 4.1	2.78; dd; 9.0; 4.0
14e	1.72; d; ~12.0	1.74; d; 12.1
14a	1.13; dd; ~12.0; 4.1	1.08; dd; 12.1; 4.0
15	4.20; br.d; 2.4	4.20; br.s
17A	5.33; br.s	5.32; br.s
17B	5.15; br.d; 2.4	5.10; d; 2.1
18	0.82; s	0.75; s
19A	3.28; d; 13.8	2.40; m
19B	3.10; d; 13.8	2.22; br.d; ~11.3
20	3.75; br.s	3.29; br.s
21A	3.24; m	2.52; m
21B	3.10; m	2.40; m
22	1.39; t; 7.1	1.03; t; 7.4

*Determined by two-dimensional homonuclear resonance (COSY).

**CH correlation (HETCOR).

The presence of the epimeric N-oxide impelled us to make a revision of the results obtained previously [1] on the biosynthesis of napelline in *A. baicalanse*. Together with napelline, we isolated a compound to which we have assigned the structure of 12-epinapelline (III) on the basis of its spectral characteristics. In its PMR spectrum, as in that of the N-oxide (I), the signal of the H-13 proton at 2.78 ppm had the form of a doublet of doublets with SSCCs of 9.0 and 4.0 Hz, which corresponds to the β-orientation of the 12-OH group, as was also shown by the ^{13}C NMR spectrum, which, in the region of rings C and D, was completely analogous to the spectrum of the N-oxide (I) and had substantial differences from napelline (Tables 1 and 2).

Stereoisomers of napelline have ben isolated previously from plamts of the *Aconitum* genus. Thus, 12-epinapelline has been detected in *A. karacolicum* [4], and 1- and 12-epinapellines are both present in *A. flavum* [3]. Napelline oxide has been

TABLE 2. Chemical Shifts of the Carbon Atoms (δ, ppm)

C atom	I	II[2]	Napelline [2]	III*
1	67.2	68.1	70.5	70.0
2	30.5	30.1	31.9	31.8
3	32.6	31.3	32.4**	36.3
4	35.2	36.2	34.7	33.8
5	46.6	47.6	49.4	48.8
6	22.8	22.9	23.6	23.7
7	46.3	47.3	45.0	44.0
8	49.8	49.8	50.3	51.0
9	39.0	39.1	38.2	37.1
10	54.2	55.5	53.5	52.6
11	28.9	29.6	29.4	29.7
12	66.6	76.6	76.2	67.2
13	43.8	48.5	49.9	44.0
14	34.9	35.3	38.4**	32.8
15	76.4	77.6	77.8	77.1
16	153.6	158.9	160.8	154.7
17	112.7	109.3	107.4	111.5
18	26.5	26.5	26.4	26.5
19	74.8	75.3	57.7	58.4
20	80.3	81.5	66.2	66.3
21	67.2	67.9	51.6	51.2
22	7.8	7.8	13.3	13.5

*Determined by the HETCOR method.

**On the basis of the results of CH correlation (HETCOR), we consider that these values in [2] and [3] should change places.

isolated not only as described in [2] but also from *A.karacolicum* [5]. So far as concerns the previous report of finding napelline in *A. baicalense*, it still remains unexplained. The suggestion of different plant chromosoms fails, since the material for the isolation of napelline was taken, even though in different years, nevertheless from the same site and at the same stage of vegetation of the plant.

EXPERIMENTAL

Melting points were determined on a Kofler stage and are uncorrectd. Vibrational spectra were recorded in KBr tablets on a UR-20 instrument. A Varian VXP-500 S spectrometer was used for recording NMR spectra. HMDS was used as standard. All the spectra were taken in deuterochloroform. Mass spectra were obtained on a LKB-2091 instrument.

We used alumina for column chromatography and for thin-layer chromatography. The plates were revealed with iodine.

Isolation of the Alkaloids. The epigeal part of *A. bacailense* (5 kg) was wetted with a saturated solution of soda and extracted with chloroform. After concentration, the chloroform extract obtained was treated repeatedly with 5% sulfuric acid. The sulfuric acid extract was neutralized with soda to pH 9-10. The alkaloids were extracted with ether and then with chloroform. After evaporation of the solvents, 4.8 g of chloroform-soluble and 6.6 g of ether-soluble bases were obtained.

The latter were dissolved in 30 ml of 5% H_2SO_4 and extracted with ether (12 × 3 ml). The fraction obtained after the ether had been distilled off was chromatographed on a column of alumina. The alkaloids reported previously [1] were obtained.

The acid solution was brought to pH 9-10 and was extracted with chloroform. The chloroform-soluble alkaloids obtained were chromatographed on deactivated alumina. Elution with chloroform yielded fraction A, enriched with component (III), while the chloroform – methanol (25:1) system gave fraction B, enriched with compound (I). When fraction A was treated with acetone a white precipitate of substance (III) was obtained. Compound (I) was obtained from fraction B in the form of the hydrochloride. This fraction was dissolved in a weak solution of acetic acid in propanol and this was mixed with an aqueous solution of ammonium perchlorate. After some time, a crystalline perchlorate separated out, and this was recrystallized from water. For subsequent investigation, the perchlorate was converted into the base, consisting of a colorless glassy substance soluble in water, alcohols, and chloroform.

12-Epinapelline N-Oxide Perchlorate, $C_{22}H_{33}O_4N \cdot HClO_4$, mp 224-225°C (water).

12-Epinapelline N-Oxide (I), $C_{22}H_{33}O_4N$, M^+ 375. IR spectrum, (ν, cm^{-1}): 3500-3350; 3000-2800; 1650; 1070; 1040; 905-920. For the ^1H NMR spectrum, see Table 1; for the ^{13}C NMR spectrum, see Table 2.

12-Epinapelline (III), $C_{22}H_{33}O_3N$, mp 163-164°C (acetone), M^+ 359. IR spectrum, (ν, cm^{-1}): 3550-3400, 3000-2800, 1660, 1100. For the ^1H NMR spectrum, see Table 1; for the ^{13}C NMR spectrum, see Table 2.

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